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A ROLE FOR EFFERENT FIBERS IN THE FROG RETINA

by

© Loren William Kline

A THESIS

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The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research, for acceptance, a thesis entitled "A Role for Efferent Fibers in the Frog Retina", submitted by Loren William Kline in partial fulfilment of the requirements for the degree of Doctor of Philosophy.

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ABSTRACT

Leopard frogs, *Rana pipiens*, were used to determine if efferent fibers in the vertebrate retina were involved in the regulation of ganglion cell firing patterns.

The output of Class III ganglion cells, recorded from the optic tectum and retina, served as controls. Flash lengths from 0.05 sec to 90 sec and of various intensities were used to stimulate the frogs. The intensities used covered the full range from scotopic to photopic levels. In a small restricted range of stimulus intensities, the controls demonstrated a rhythmic bursting firing pattern. This occurred primarily at the offset of a mesoptic level stimulus. If the efferent fibers, within the optic nerve, were involved in the regulation of the bursting pattern, a change in the output of the Class III cells might occur when the retina was isolated functionally from the brain. Two experimental procedures were performed to determine whether or not a change occurred. A reversible cold block was used to block the optic chiasma in one series. In the second series, the optic nerve or chiasma was severed with a heater wire.

The latencies and durations of the experimental responses were compared statistically with the control responses which were obtained using identical stimuli. No statistically significant difference was noted in any case. However, one consistant difference was observed. The rhythmic bursting firing pattern disappeared whenever the optic nerve was blocked or severed. The rhythmic res-

ponse was replaced by a mass of spikes similar to those obtained from scotopic level stimuli. Since the rhythmic pattern disappeared when the nerve was blocked, it was concluded that the efferent fibers in the optic nerve must play a role in the regulation of the retinal ganglion cell output. It was speculated that the effect of the efferent fibers was mediated through the amacrine cells.

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INTRODUCTION

Hartline (1938, 1940) was the first to study the discharge of impulses in single optic nerve fibers of cold-blooded vertebrates, in response to illumination of the retina. He found that the frog ganglion cell responses fell into three categories. Each fiber displaved only one of the three response categories. The first category was termed ON fibers. These fibers started to fire at the onset of illumination with a burst of high frequency spikes which was followed by a slower steady discharge throughout the duration of illumination. The second class responded to the onset and offset of illumination, but not to steady illumination. These were termed ON-OFF fibers. The third class of cells did not respond at all during illumination, but responded with a vigorous discharge when the illumination was extinguished.

This work was extended by Maturana *et al.* (1960). These workers found that the ON fibers of Hartline could be divided into two classes, depending upon how the cells responded to a small moving spot. Class III cells were the same as the ON-OFF fibers. These were also found to be movement sensitive. The OFF fibers of Hartline were identical with the Class IV cells of Maturana.

Neither Hartline nor Maturana studied in detail any of the effects of the background illumination on the firing patterns of the ganglion cells. Hartline did note that the response was consistent in both light and dark adapted conditions, but he failed to note any difference in the pattern of the response. Pickering

and Varju (1969) made some observations which indicated that background illumination does affect the firing pattern of the ganglion cells. Under photopic conditions, the duration of the ON and OFF responses of Class III cells were of approximately equal duration. The latency of the ON response was longer than for the OFF response. The latencies of both responses became longer with decreasing stimulus intensities. In dark adapted conditions, at low stimulus intensities, the OFF stimulus elicited more spikes than the ON stimulus. Thus, it can be seen that the level of background illumination does affect the firing patterns of Class III cells.

One of the oldest and most controversial aspects of visual physiology concerns the existence and function of efferent fibers to the retina. Their existence in the optic nerve of pigeons and chickens has been fairly well established (Dowling and Cowan, 1966; Holden, 1968a; Holden and Powell, 1972; McGill *et al.*, 1966a, b; Miles, 1970, 1972a, b, c, d; Rogers and Miles, 1972). However, in the frog the evidence for their presence is not as well established (Cajal, 1952; Branston and Fleming, 1968). The situation for mammals is still unclear. In birds, where the efferent fibers are known to exist, little work has been done as to their possible function. Anatomical evidence indicates that efferent fibers in the birds and frogs seem to be associated with the amacrine cells of the retina. It has also been shown, in the frog, that nearly all ganglion cells make synaptic connections primarily with amacrine cells. Thus, nearly all information travelling vertically from the bipolar cells to the ganglion cells must "pass through"

these cells (Dowling, 1968). Little is known of the function of efferent fibers to the retina.

Since little is known of the effects of stimulus intensity levels on the ganglion cell firing patterns, the following experiments were designed to determine if efferent fibers to the retina play a role in the regulation of ganglion cell firing patterns.

LITERATURE REVIEW

I. Class III Cells

Hartline (1938, 1940) studied the discharge of impulses in single optic nerve fibers of the frog eye, in response to illumination of the retina. It was found that 20% of the fibers studied responded only to the onset of illumination. These cells respond with an initial burst of impulses at high frequency. This was followed by a steady discharge at a lower frequency which lasted throughout the duration of the illumination. These have been termed ON fibers. A second type of response occurred in 30% of the fibers studied. The fiber was inactive when the retina was illuminated. When the illumination was extinguished, a vigorous discharge occurred. This discharge lasted several seconds and usually subsided gradually. These have been termed OFF fibers. The last category described comprised 50% of the fibers studied. These fibers responded with a short burst of impulses at high frequency when the light was turned on, but show no impulses as long as it continues to shine steadily. When the light was extinguished, another short burst of impulses occurred. These cells have been termed ON-OFF fibers. Hartline made a brief study of the fibers in dark adapted conditions; however, it was found that the sensitivity of the fibers increased as the retina dark adapted. No changes in firing patterns was described. The fibers still responded in the same manner to the same stimuli.

Maturana *et al.* (1960) studied the output of the retinal

ganglion cells of *R. pipiens*. Recordings were made from both single optic nerve fibers as well as the terminal arborizations of the optic nerve fibers in the optic tectum. A comparison of the results from the two sets of recordings indicated that there was no difference in the responses recorded. Thus, since it was easier to record from the optic tectum, the majority of their study was carried out using recordings from the tectum. Five categories of cells were observed in both the optic nerve fibers and the optic tectum. It should be noted that while recording from the single nerve fibers, the different cell types were encountered randomly. In the tectum, however, it was found that each cell type was arranged in a sequential layered manner. The layering was in the stratum album centrale of the superficiale optic tectum. The operations performed by the five classes of ganglion cells were as follows. Class I and II cells were found to respond to edges in the ganglion cell's receptive field (RF). The RF of a single ganglion cell was defined by Hartline (1938, 1940) as the area of the retina within which stimulation causes it to discharge. Class I and II cells were subtypes of Hartline's ON fibers. The difference between Class I and II cells was that Class I cells responded to the edge as it moved into the RF and stopped. If the background illumination was extinguished, the cell stopped firing. When the background illumination was turned on again, the cell started to respond again after a short pause. This property was called "nonerasibility". Class II cells were erasible, i.e., they would not respond again after the background

illumination was turned on again, unless the edge moved again. Class IV cells were found to respond to any adequate darkening of the RF with a prolonged off discharge. They also responded to a moving object in proportion to the percentage of darkening that the object produced during movement. These cells were identical to the OFF fibers of Hartline. Class V cells were termed darkness detectors. These cells responded continuously in the dark and ceased when illuminated. These cells were fairly rare. Class III cells were found to be identical to the ON-OFF fibers of Hartline. These cells were also found to be movement sensitive and were not concentrically organized as the RFs of the cat. These workers did not study the effects of the background illumination on the firing pattern of the cells described.

Grusser *et al.* (1964, 1967) studied the response of Class III cells. These workers studied the effects of several parameters on the response of Class III cells. These parameters included angular velocity, stimulus size, contrast of the stimulus, position of the path through the RF, and the time between the movement of identical stimuli along the same path through the RF. The response of the Class III cells was dependent on some of the physical parameters. The average luminance of the stimulus pattern, if changed within the photopic range, was the only one which seemed not to influence the response. The other parameters all had an effect on the response of the cells. However, the study was only concerned with photopic levels and no study was made to determine if the relationships described varied with changes in the background

illumination.

Pickering and Varju (1969) studied Class III cells of the frog. These workers were primarily interested in the occurrence of a delayed response of ganglion cells. They found that a very brief intense flash presented to a dark adapted retina could generate activity which appeared as long as 20 sec after the stimulus. These workers also observed that under light adapted conditions, the ON and OFF responses of Class III cells were of approximately equal duration. However, the latency of the ON response was longer than for the OFF response. The latencies of both ON and OFF responses became longer with decreasing stimulus intensity. In dark adapted conditions at low stimulus intensities, the OFF stimulus elicited more spikes than the ON stimulus. This was the first report that a change occurred in the firing pattern of Class III cells as the frog dark adapted.

Barlow *et al.* (1957) had observed changes in the RF organization of the cat as it was dark adapted. Under photopic conditions, a majority of the RFs in the cat retina are concentrically organized. The annulus surrounding the RF center either depresses or completely inhibits the center's response when both are stimulated simultaneously. Barlow *et al.* studied off-center ganglion cells, i.e. ganglion cells which responded when a spot of light, in the field center, was extinguished. The cat was dark adapted. A spot of light was presented to the center and the expected off response occurred when the spot was extinguished. The spot's diameter was gradually increased. No antagonistic effect was noted.

This indicated a reorganization of the RF occurred during dark adaptation. Thus, it was shown that changes can occur in ganglion cell responses when the retina it adapted to different background illuminations.

Sakmann and Creutzfeldt (1969) studied the effect of background illumination on the response of on-center ganglion cells of the cat. These workers used backgrounds in the scotopic-mesoptic range (10^{-5} to 1 cd/m^2). The maintained discharge rate of all on-center units was found to increase with increasing adapting luminances up to 10^{-2} cd/m^2. Above this luminance, the discharge leveled off or decreased. The change of the maintained discharge rate with increasing adapting luminances was found to be related to changes in the RF organization and to changes in retinal sensitivity. Again, it has been shown that changes occur in the response patterns of ganglion cells under different levels of adaptation. Thus, it has been shown that while a ganglion cell may respond to only one stimulus modality, the nature of the firing pattern depends, in part, upon the adaptational state of the retina.

II. Oscillatory Potentials in Vertebrates

The occurrence of periodic oscillations of potential in the visual system was first observed in the frog by Gotch (1903) and Einthoven and Jolly (1908). Gotch observed, while recording with large gross electrodes on the optic nerve, that the presence of the oscillations depended on previous light experience. The response had a greater amplitude if the retina had previously been in the

dark. Einthoven and Jolly used several intensities of light to stimulate the oscillations. They observed that the offset of a stimulus of one intensity produced rhythmic oscillations. Chaffee and Sutcliffe (1930) also observed oscillations from the optic disk of the frog. Volkmer (1956/57) found that in the frog the frequency of the rhythmic oscillations remained constant despite great changes in light intensity. He also observed that the oscillations could be detected with stimuli too dim to elicit the usual electroretinogram (ERG).

While all the previous workers used gross recording techniques, some intraretinal recordings were made by Tomita and Fumaishi (1952) and by Brindley (1956). Tomita and Fumaishi used the bullfrog, while Brindley used the frog. These workers attempted to locate the origin of the oscillatory potentials. Both groups of workers observed that the oscillations were largest in the region of the bipolar and amacrine cells.

Oscillations have been observed in the ERG of several other animals. Granit (1933) was the first to observe oscillations in the retina of mammals. He recorded from the optic nerve of decerebrate cats. The frequency range for these oscillations ranged from 100 to 150/sec for on and off responses. The oscillations were abolished by ether anaesthesia or carotid occlusion. As in the frog, these oscillations changed in amplitude, but not in frequency, with changes in the light stimulus intensity. Dodt and Wirth (1953) studied the ERG of pigeons. Oscillations at 100/sec were observed. These were similar to those observed in frogs.

Yonemura *et al.* (1963) and Yonemura and Hatto (1966) studied the oscillations in the frog ERG in more detail. The rhythmic oscillations were found to occur on the ascending branch of the b wave. The period of the oscillations in the frog was 45/sec. A study was also made of the cat, rabbit, guinea pig, pigeon, chicken and tortoise ERGs. The rhythmic oscillations were present in the ascending slope of the b wave in each case. The frequency of the oscillations varied from species to species. These workers also showed, in the frog, that the frequency of the oscillations was independent of the stimulus intensity. The origin of the oscillations was found to be near the bipolar cell layer. The authors concluded that the oscillations had an origin different from the generating mechanisms of the classical ERG and was a distinct component of it. They did not speculate as to the functional significance of the oscillations.

Graham and Pong (1972) observed rhythmic oscillations in the rat ERG. These oscillations had been shown by Brown and Rojas (1965) to be prominent in the rat retina after barbiturate anaesthesia. Graham and Pong studied the effects of various drugs on the ERG rhythmic oscillatory potentials. They determined that GABA could be an inhibitory transmitter from a cell which was responsible for the potentials. They concluded that an inhibitory input by the amacrine cells to the bipolar and ganglion cells caused the observed rhythmic oscillatory potentials. However, the results are based on recordings made from the surface of the cornea. No single unit recordings were made. They also assumed that the amacrine cells were

inhibitory. However, the amacrine cells may be inhibitory as a result of an inhibitory input to them. This source of input to the amacrine cells could be the source of the rhythmic potentials. Algvere and Wachtmeiser (1972), Algvere *et al.* (1972), and Algvere and Westbeck (1972) studied the oscillatory potentials in the human retina. These had been first described by Cobb and Morton (1954). Cobb and Morton noted that short light flashes of high luminance stimulated oscillatory components of the b wave of the ERG. Algvere and his various co-workers studied the phenomenon in more detail. They observed that the oscillatory potentials vanished during the rod phase of dark adaptation. The disappearance always followed a pattern. The light adapting effect of a flash enabled oscillatory potentials to be observed for about 1 minute, if the flash was delivered in the rod phase of dark adaptation. During the cone phase of dark adaptation, the oscillations were regularly observed. A fairly constant frequency of between 120 to 130 Hz was observed. It was also observed that the stimulus intensity affected only the amplitude of the response, but it did not affect the frequency. These workers concluded that the origin of the oscillations was independent of the processes contributing to the a wave and b wave of the ERG. The authors further noted that "the oscillations sensitively reflected the great changes in retinal adaptation". Since the oscillations occur in a light adapted retina and disappear when the retina is dark adapted, the authors concluded that reorganizational changes must occur in the retina which either permit or inhibit the oscillations. They felt it was not unreasonable

to assume that the oscillatory potentials may "reflect some activity from the amacrine cells".

Doty and Kimura (1963) studied the oscillatory potentials in the visual system of cats and monkeys. These workers recorded from the optic nerve using large bipolar electrodes. They found that the rhythmic waves occurred in response to bright flashes. The frequency of these oscillations was independent of the intensity of the stimulus. Monkeys displayed a rhythmic frequency of from 50/sec in deep barbiturate anaesthesia to 160/sec in chronically prepared, unanaesthetized monkeys. These authors examined the possible origins of the oscillatory potentials. It was determined that the oscillations were not due to electronic spread of postsynaptic potentials. There was no attenuation in the amplitude of the oscillations with distance from the retina as would be expected if electrotonic spread were the cause of the oscillations. Thus, these workers concluded that the oscillations must be propagated. These workers also studied the effects of antidromic stimulation of the optic nerve on the oscillatory potentials. They found that intense antidromic stimulation did not reset the rhythm elicited by the flashes. It was inferred that the rhythm was not set by the ganglion cells. The electrical stimulation of the intact nerve, while not resetting the rhythm, was able to elicit it. It was unlikely that stimulation of the optic nerve could elicit rhythmic activity solely by antidromic invasion of the ganglion cells; and retinal nerve fibers lack collaterals (Cajal, 1955) to effect more complex connections. Centrifugal fibers which seem to end near amacrine cells were speculated to have access to the rhythmic

generating mechanisms.

Steinberg (1966) studied the oscillatory potentials in the retina of cats. He observed that light adaptation enhanced the development of oscillatory activity stimulated by short duration flashes. A retina which had been dark adapted, following a period in the light, was unable to generate oscillatory potentials which could follow short flashes presented at fixed intervals. A low intensity flash could not stimulate oscillatory potentials in a dark adapted retina. When the retina was "in a state which could generate the potentials", a frequency of 80-120/sec was observed. This frequency was unaltered by flash rate or flash intensity. Steinberg demonstrated that the oscillatory potentials were due to the synchronous discharge of the ganglion cells. He concluded that a retinal mechanism, producing high frequency cyclical facilitation-inhibition of ganglion cell discharge, was probably related to a process of neural light adaptation.

Laufer and Verzeano (1967) studied the oscillatory potentials in the cat visual system. The oscillatory potentials and the bursting pattern observed was also called periodic activity.

Oscillatory potentials were observed in unanaesthetized cats, which had been kept in darkness, in response to the turning on of a light stimulus. The regular oscillations had a frequency range from 40-100/sec. In a given animal, the frequency range was the same for all illuminations tested. This range extended from scotopic to photopic levels. The oscillations showed a tendency to periodically wax and wane in amplitude. A decrease in amplitude

was observed after 5-10 sec and increased again after 30 sec of continuous illumination.

Under the same conditions, a microelectrode was placed in the ganglion cell layer of the retina. A series of periodic bursts of spikes was recorded. After a latent period, an initial discharge occurred. This was followed by clear bursts of spikes separated by silent periods. The frequency of the bursts was the same as that of the optic nerve and tract oscillations. Recordings of retinal spikes and the optic tract oscillations were made simultaneously. A burst of neuronal spikes in the retina was found to correspond to each positive peak of the optic tract oscillations.

Similar experiments were performed to examine the oscillations produced in response to the extinguishing of a given level of illumination. A regular sequence of oscillations was observed after the extinguishing of the light. The frequency ranged between 20-40/sec and was not dependent upon the previous level of illumination. The amplitude and the length of time the oscillations occured was, however, related to the previous level of illumination. In darkness, as in light, the neuronal activity in the retina again consisted of bursts of spikes. The bursts of spikes in the retina corresponded to each positive peak of an oscillation in the optic tract.

Previous experiments using single cell recordings (Kuffler *et al.*, 1957; Fuster *et al.*, 1965) questioned the existence of periodic discharge in retinal neurons. Laufer and Verzeano noted that a sequence of spikes generated by a single cell demonstrated

little rhythmicity. However, their data indicated that the response of groups of several cells could maintain highly periodic activity and could maintain consistent phase relations with the periodic oscillations recorded from the optic tract. Analysis of their data indicated that there was little variation in the size of the interburst intervals and that the occurrence of the bursts of multineuronal spikes was highly periodic. It was also found that periodic oscillations in the optic tract were always associated with bursts of neuronal spikes in the retina and in the optic nerve.

Oscillations similar to those found in the retina and optic tract have been found in the thalamus and cortex. In both the thalamus and cortex, bursts of spikes were associated with oscillations. A constant phase relation existed between the two. An increase in the amplitude of the oscillations corresponded to an increase in the clustering and number of spikes in the burst (Verzeano and Calma, 1954; Verzeano, 1955; Schlag, 1956). The same relation was found to be true for the retina.

Laufer and Verzeano (1967) cut the optic tracts in order to determine if efferent fibers played a role in the origin of the bursting pattern of neuronal activity. The severance had no effect on the oscillatory potentials. However, it is possible that the efferent fibers join the optic nerve at the chiasma and not in the tract. This has been shown to be true in the frog (Larsell, 1924; Lazar, 1969) and pigeon (Galifret *et al.*, 1971). Thus, it is still possible that efferent fibers may be involved in the generation of the oscillatory potential.

These workers concluded that the periodic activity of the retina might be involved in the regulation of the sensitivity of the retina.

It should be noted, briefly, that two central visual areas also exhibit oscillatory potentials. Hughes and Mazuroski (1962) observed rhythmic oscillatory potentials in the visual cortex. The potentials appeared as wavelets occurring every 5-7 msec. Doty and Kimura (1963) also observed these oscillatory potentials in the monkey striate cortex. Verzeano (1955) and Neigishi, Lu and Verzeano (1962) observed that periodic bursts of spikes and periodic oscillations developed spontaneously in the lateral geniculate body (LGB) of the cat. Laufer and Verzeano (1967) found, by recording simultaneously from the retina and LGB, that the spontaneous activity of the LGB was suppressed when the activity related to the retina developed. The spontaneous activity reappeared when the retinal activity ended. These workers concluded that this process of interaction between the activity of the retina and LGB could be "the forwarding of information concerning the distribution of excitation and inhibition in the retina and about the condition of light and darkness in the environment".

Thus, it can be seen that periodic oscillatory potentials occur in the retina, optic nerve, and optic tract of many species, including the frog. Further, it has been shown that the oscillatory potentials are due to clustered bursting activity of retinal neurons. It has been speculated that this activity could control retinal sensitivity as well as relay information concerning light and darkness

in the environment to the brain.

III. Efferent Fibers in the Vertebrate Retina

Histological evidence for efferent fibers and for retinal components of an efferent system has been provided by Dogiel in the chick (1895), Cajal in the chick (1889), Johnston in the pigeon (1906), and Polyak in the chimpanzee (1941). Honrubia and Elliott (1968, 1970) demonstrated efferent fibers in human and monkey retinas. Gills (1966) supported the findings of Honrubia and Elliott by also observing efferent fibers to the human retina. Cajal (1952) believed that fibers to the retina mediated control over visual sensitivity. Perlia (1889), using the chick, provided experimental evidence to support the findings of Cajal and Dogiel. Perlia enucleated one eye of a newborn chick and observed, several months later, a distinct cell mass, the isthmo-optic nucleus (ION), was totally atrophied. Wallenburg (1898) supported the observations made by Perlia. Wallenburg showed that after lesions were made in the ION, degenerating fibers, stained by the Marchi method, could be followed rostrally in the isthmo-optic tract (IOT), across the optic chiasma, and through the medial part of the contralateral optic nerve in the nerve fiber layer of the retina. Here they spread out to all parts of the retina and seemed to end in the vicinity of the ganglion cells. Cowan (1970) and Cowan and Powell (1963) repeated the work and demonstrated that the efferent fibers composed about 1% of the fibers in the pigeon optic nerve. The fibers were found to cross the inner plexiform layer to their terminations along the inner aspect of the inner nuclear layer, where

they ended on the amacrine cells. There was no evidence that they ended on any ganglion cells.

Arey (1916) obtained physiological evidence for efferent functioning during retinal light and dark adaptation in the fish, *Ameiurus*. Arey observed that in a light adapted retina, the "retinal pigment (choroid) surrounds the visual cells (rods and cones)". When the animals were allowed to dark adapt, the "pigment cells contracted away from around the visual cells". When one optic nerve was severed proximal to the chiasma, the retinal pigment in the contralateral eye failed to undergo the usual changes as the animal was light and dark adapted. The pigment remained distributed as it would normally appear in a dark adapted eye. When the retina was hemisected, one-half of the eye responded normally while the other half failed to respond. Arey concluded that efferent fibers from the brain to the retina must mediate the migrations of the retinal pigment. Further evidence of a possible role of efferents was observed by Motokawa and Ebe (1954). They demonstrated that antidromic stimulation of the optic nerve depressed the sensitivity of the cat retina to stimuli. They speculated that this depression of sensitivity could play a role in adaptational changes in the retina. Jacobson and Gestring (1958) also studied a possible role of efferents in the cat retina. They studied changes in the cat and monkey ERG with dark adaptation and the administration of drugs. These workers found evidence that efferent fibers in the optic nerve play an active role in dark adaptation. Their results supported their hypothesis that, "a center in the brain exists which

they ended on the amacrine cells. There was no evidence that they ended on any ganglion cells.

Arey (1916) obtained physiological evidence for efferent functioning during retinal light and dark adaptation in the fish, *Ameiurus*. Arey observed that in a light adapted retina, the "retinal pigment (choroid) surrounds the visual cells (rods and cones)". When the animals were allowed to dark adapt, the "pigment cells contracted away from around the visual cells". When one optic nerve was severed proximal to the chiasma, the retinal pigment in the contralateral eye failed to undergo the usual changes as the animal was light and dark adapted. The pigment remained distributed as it would normally appear in a dark adapted eye. When the retina was hemisected, one-half of the eye responded normally while the other half failed to respond. Arey concluded that efferent fibers from the brain to the retina must mediate the migrations of the retinal pigment. Further evidence of a possible role of efferents was observed by Motokawa and Ebe (1954). They demonstrated that antidromic stimulation of the optic nerve depressed the sensitivity of the cat retina to stimuli. They speculated that this depression of sensitivity could play a role in adaptational changes in the retina. Jacobson and Gestring (1958) also studied a possible role of efferents in the cat retina. They studied changes in the cat and monkey ERG with dark adaptation and the administration of drugs. These workers found evidence that efferent fibers in the optic nerve play an active role in dark adaptation. Their results supported their hypothesis that, "a center in the brain exists which

provides a feedback control that regulates the rate of retinal activity and controls dark adaptation". Brindley and Hamasaki (1962a, 1966) disagreed with the findings of Jacobson and Gestring. These workers did two experiments. The first was a histological study and the second was an electrophysiological one. They used a Nauta-Gygax silver stain during their experiments on axonal degeneration of the cat optic nerve. It has since been shown that certain parts of the central nervous system cannot be stained by silver methods, but it is possible to recognize them with the electron microscope (Boycott *et al.*, 1961; Colonnier, 1964; Gray, 1962). Thus, it is possible that the negative histological results of Brindley and Hamasaki could be due to the use of an inadequate staining procedure. Brindley and Hamasaki (1962b) also presented some physiological evidence that centrifugal fibers did not exist in the cat optic nerve. They did not observe any changes in the amplitude of the ERG immediately after section of the optic nerve as had Jacobson and Gestring. It should be mentioned that Brindley and Hamasaki did not experiment in as much detail as Jacobson and Gestring. The lack of reproducibility does not either confirm or deny the existence of centrifugal fibers in the cat's retina. It does suggest that both sets of experiments should be carefully repeated.

Brooke *et al.* (1965) sectioned the optic nerve of the cat at its junction with the optic chiasma. After survival periods varying from 6 to 14 days, the animals were anaesthetized. The eyes were removed and prepared for electron microscope study according to

the Nauta method. Fibers were found which demonstrated the same indications of degeneration as efferent fibers in the pigeon. The fibers contained whorls of electron dense membranes and had numerous shrunken, opaque mitochondria. These degenerating fibers were followed. They were found to extend between the cell bodies of the ganglion cells and into all levels of the inner plexiform layer. The degenerating fibers were found in relation to the cell bodies of amacrine cells. The degenerating fibers were considered to be efferent fibers to the retina. The findings were considered valid since the observed changes in the optic nerve and inner plexiform layer of the retina were similar to those described in other sites of the central nervous system and the peripheral nervous system. The findings in the cat were the same as in the pigeon which is well known to contain efferent fibers to the retina.

Dodt (1956) found that after a single electrical stimulus to the rabbit optic nerve or tract, the antidromic volley recorded from the optic nerve head was followed by delayed spikes. These spikes occurred from 7 to 25 msec after the electrical stimulus. A second characteristic of the delayed spikes was that unlike the antidromic volley, the delayed spikes quickly habituated. It is of interest to note that Miles (1972b) found that the efferent system of the pigeon also quickly habituates. Dodt found that the delayed spikes were also dependent upon the level of adaptation of the retina. The delayed spikes occurred only when the retina had completely adapted to either the photopic or scotopic levels. Unlike the antidromic activity which was always present, the delayed spikes were

absent while the process of adaptation was taking place. A fourth characteristic of the delayed spikes was that they could not be elicited by light flashes of any intensity or wavelength. Dodt concluded that the delayed spikes were postsynaptic activity recorded from centrifugal fibers to the rabbit retina.

Dowling and Cowan (1966) have examined the centrifugal fibers in the pigeon retina. These workers have shown that the efferent fibers make synaptic contact with amacrine cells. This finding has been supported by the work of Maturana and Frenk (1971). Thus, it can be seen that an anatomical pathway exists whereby efferent fibers may influence amacrine cells which in turn could regulate the activity of ganglion cells. Some workers also believe that the efferent fibers also regulate some of the neural changes occurring during dark adaptation.

The efferent system has been thoroughly investigated in the pigeon and chick (Holden, 1968a, b; Holden and Powell, 1972; McGill *et al.*, 1966a, b; Miles, 1970, 1972a, b, c, d; Rogers and Miles, 1972). Miles (1972b, c) and Pearlman (1973) have shown that a closed loop exists in the pigeon and chick retina. These researchers have demonstrated that the output of the ganglion cells feeds both the optic tectum and the ION. The ION sends fibers to the amacrine cells. The efferent fiber input to the amacrine cells may cause a modification of the ganglion cell output. Miles (1972a, b, c, d), using the chick, and Pearlman (1973), using the pigeon, have also shown that the primary action of the efferent fibers in the pigeon and chick retina is that of disinhibition to the inhibitory sur-

rounds to the on-off, movement-sensitive RFs. These workers believed that the efferent fibers turn off the inhibitory input of the amacrine cells to the ganglion cells, thereby causing the increased size of the excitatory RF. When the experiments were repeated using an intact animal, identical stimuli, and a reversible cold block on the IOT, such findings were not obtained. In fact, the data obtained offered no support to the hypothesis. The data obtained from the reversible cold block experiments indicated "no clear cut effects on the visual responses of retinal units to a variety of moving targets or to stationary spots of light of various sizes turned on and off in their RF centers". No attempt was made to determine if the centrifugal system played a role in the regulating of the ganglion cell output at different levels of adaptation.

Rogers and Miles (1972) also studied the effect of lesions to the IOT on the normal behaviour of chicks. It was found that the chicks failed to perform discriminatory tasks in situations of reduced background illumination or contrasts in the background. For example, the chicks were unable to discriminate between kernels of grain and pebbles when placed on a black and white checkerboard background. This study concluded that on the basis of the cold block and behavioural studies, the efferent fibers might play an important role in the adapting of the retina to different background illuminations.

While the situation for the chick and pigeon has been worked out fairly well, much less is known as to the role of efferent fibers in the frog. Efferent fibers have been shown anatomically to

exist in the toad and frog (Cajal, 1952; Lettvin *et al.*, 1961; Maturana, 1958a, b). Further, it has been shown by Larsell (1924) and by Lazar (1969) that a fiber pathway connects the optic tectum with the isthmo-optic nucleus. They also observed that the ION sends fibers to the optic tract. These fibers cross over at the chiasma and enter the contralateral eye with the optic nerve. Dowling (1968) observed that amacrine cells make extensive synapses with the ganglion cells. In fact, Dowling has shown that almost all input to the ganglion cells must go through the amacrine cells. Synaptic connections between efferent fibers and amacrine cells have been observed (Cajal, 1952). This indicates that an anatomical loop similar to that described in the pigeon and chick exists in the frog. In addition to this anatomical evidence for efferent fibers, some physiological evidence also exists. *Rana pipiens* have movement-sensitive, on-off cells in the retina which are similar to ones in the chick and pigeon (Grusser and Grusser-Cornehls, 1964; Grusser *et al.*, 1967; Maturana *et al.*, 1960). This type of cell has been shown to be regulated by amacrine cells and thus could, in turn, be regulated by efferent fibers. Branston and Fleming (1968) recorded from efferent fibers in the frog optic nerve. However, the work concentrated on the measuring of efferent activity in response to extravisual stimuli. Efferent activity was also measured in the proximal stump of the cut optic nerve. The workers also examined the effects of extravisual stimuli on RF size. They noted that the inhibitory RF increased in size with audio stimuli. They also mentioned that the retina underwent adaptational changes, but did

not mention specific changes. However, when the optic nerve was cold blocked in intact animals, the results lent some support to the hypothesis that the efferents provided a "counter-inhibitory" effect. Thus, in the frog little is known of the possible role of efferent fibers to the retina. It would be of interest to determine if the efferent fibers in the frog play any role in regulating the firing pattern of ganglion cells.

METHODS

I. Animal Preparation

A. Care

The leopard frog, *Rana pipiens*, was obtained from a commercial supplier. The frogs were kept in the University of Alberta Health Sciences Animal Center. Two weeks prior to use, a half dozen frogs were removed to the laboratory and kept in a large pen. Two trays of water were kept in the pen, and were changed daily. The frogs were fed meal worm beetles, *Tenebrio molitor*, daily. Any frogs showing signs of red leg of other damage were discarded.

B. Surgery

Two different surgical procedures were performed, depending on the site for recording. In the first series, which acted as controls, recordings were made from the right stratum album superficiale of the optic tectum (Maturana *et al.*, 1960). The frogs were anaesthetized by immersion in 0.1% tricaine methanesulfonate (Kaplan, 1969). Since all the recordings were made on awake, alert frogs, it was necessary to inhibit as much movement as possible; therefore, the two sciatic nerves were severed above the level of the pelvic girdle. The two brachial nerves were severed at the shoulder. In order to reach the optic tectum, a midline incision through the skin from the level of the eyes back to approximately the second cervical vertebrae was made. The occipital artery was dissected free and moved laterally

in order to provide access to the fronto-parietal and exoccipatal bones (Ecker, 1889). This and following procedures were performed with the aid of a dissecting microscope. These bones were carefully removed using a high speed dental drill. The dura mater was carefully snipped away, leaving the right lobe of the optic tectum exposed. The tectum was covered with parafin oil. During the surgery excessive bleeding was avoided. If it occurred, the animal was discarded.

C. Care during recording

It has been observed that it was difficult to obtain good results from experiments performed during late spring and summer. Since the techniques used throughout the experiments were identical, the source of the problem should be with the frogs. Since the frogs were immobilized and in some experiments the lower jaw was pinned underneath the animal, normal breathing movements were impossible. The only oxygen available to the frog was that absorbed through the skin. It was decided to determine whether or not hypoxia could account for the seasonal variation. This hypothesis was tested in the following manner. The frogs were surgically prepared in the usual manner. They were pinned to a corkboard which had holes cut in it in the area covered by the frog. The cork was placed in a metal box which had a plastic tube running along the bottom center of the box. Another tube, continuous with the first, ran at right angles to the one running lengthwise. This system had small holes bored into it. The tube was connected to a nipple on the back of

the box. This tubing system provided a means of providing an oxygen mixture to the frog. The mixture used was 95% oxygen - 5% CO_2 (carbogen). After the usual dark adaptation period, a few recordings were made in the usual manner. The carbogen was turned on for 5 minutes. Identical recordings were made and compared. It was found that the responses observed during the application of the carbogen were similar to those made during the winter. The administration of an enriched atmosphere to the frog was supported by the work of Fromm and Johnson (1955). They found that the oxygen requirements of *Rana pipiens* in the spring and summer was six times the winter needs. These workers suggested that the administration of an enriched atmosphere alleviated the problem of hypoxia. Carbogen was administered throughout all of the experiments for which data are reported here.

The frog was covered with a damp surgical sponge to prevent drying. The frog and box were placed on a special table for recording. The table top could be rotated 360°, and a scale was marked on the table to determine the amount of rotation. The top could be raised or lowered hydraulically. Lastly, the table top could be tilted up or down 30°. An adjustable point above the center of the top remained fixed as the top was tilted. This point was adjusted to the height of the frog's eye above the table top. This feature made the locating of a RF less difficult. It also made the presentation of the stimulus to a RF easier.

The nictating membrane of the left eye was carefully removed. A syringe pump and tube presented the eye with a drop of water every

minute. This prevented the drying of the cornea. If the cornea began to dry, it became cloudy and the frog had to be discarded.

The surgical procedure for the retinal controls and the two experimental series differed slightly from that followed for the tectal recordings. In these experiments the four nerves were cut as previously described. A piece of skin over the left eye was removed. Next, a small hole was cut in the sclera, carefully avoiding the puncturing of the choroid or retina. This allowed the introduction of the electrode into the eye without either damaging the electrode tip or producing such a large aperture that the eyeball deflated. In the two experimental series an additional procedure was performed. The optic chiasma was exposed by carefully removing most of the parasphenoid bone and part of the cartilaginous cranium with a dental drill. The chiasma was covered with parafin oil. This opening allowed the introduction of either the cryoprobe or a heating wire to the optic chiasma.

The frog box was also modified for this experimental series. Since the cryoprobe was to be introduced onto the optic chiasma from below, the box had legs 12.5 cm long mounted onto it. Access to the ventral surface of the skull was obtained by pinning the frog to the box in such a manner that the upper jaw overextended the side of the box. The lower jaw was held under the frog's body. In order to hold the head securely, a plastic holder, shaped like the outline of the upper jaw, was glued to the edge of the box. A dab of moldable dental silicon impression compound was placed on the apex. This permitted the pinning of the jaw to the holder. In order to observe

the optic chiasma and to place the cryoprobe or heater wire in position, a mirror was placed underneath the box at an angle of 120°. The image of the plastic jaw outline and the ventral surface of the skull were then visible in the mirror. The ventral surface of the jaw was illuminated with a fiber optics light source and guide. The image of the optic chiasma was magnified using a Zeiss Operation Microscope. The probe or wire could then be placed in position by indirect observation of the chiasma and probe or wire images (Figure 1).

II. Experimental Procedure

A. Recording procedures

All the recordings were extracellular. The microelectrodes used had tip diameters of 2 to 5 μ. After pulling the electrodes, they were filled with Wood's metal and the tip electroplated with a thin layer of gold (Dowben *et al.*, 1953). The electrode tips were then electroplated with a thin layer of platinum black (Gestland *et al.*, 1956) and inspected. The tip was measured with an eyepiece micrometer and the quality of the platinum coat was determined. If the tip had a ball shaped end, it was discarded. This type of tip easily broke off and rendered the electrode unusable. A sheath coating which conformed to the shape of the tip was found to work best.

The electrodes were introduced into the optic tectum with the aid of the operation microscope. The electrode was lowered to just above the tectum with a micromanipulator and placed in the

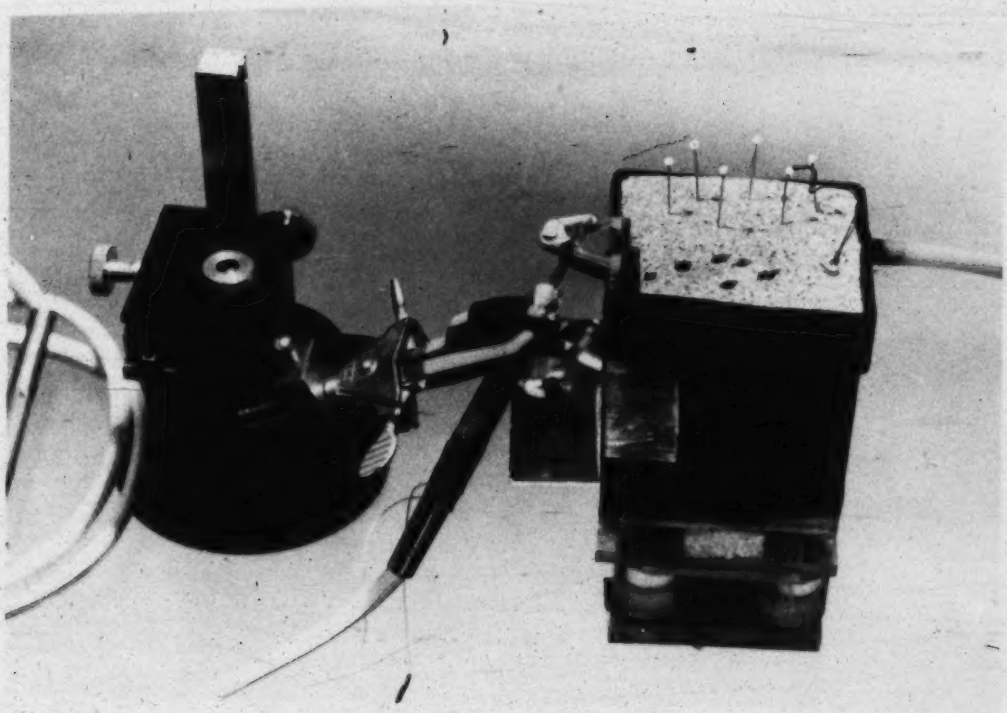


Figure 1. Frog box and cryoprobe used in cold block experiments.

tectum using a hydraulic microdrive. All blood vessels and pigment spots on the brain were avoided. The blood vessels were avoided to prevent a hemorrhage and the pigment spots were avoided as they were tough and would break the electrode tip if penetration were attempted.

After the microelectrode had been placed in contact with the tectum, the search for an On-Off cell began. The search consisted of flashing the light source onto the half ping pong ball screen. The light source used throughout all the experiments consisted of a 12 v automobile lamp in a lamp housing. An electronic shutter was mounted on the front of the housing. The shutter had an iris diaphragm built in, thereby allowing the diameter of the beam to be adjustable. A holder was mounted on the front of the shutter. Neutral density filters were placed in the holder as required. These filters and the light source were calibrated by Technical Services. A beam splitter was also placed in the system. This allowed part of the beam to be directed to the phototransitor light detector which was used to determine the onset and offset of the stimulus. Finally, a half ping pong ball acted as an evenly illuminated screen behind which the frog was placed (Figure 2). As the electrode was lowered through the layers of the tectum, the output of any cells near the electrode tip were visually monitored on a cathode ray oscilloscope. An audio amplifier allowed auditory monitering. The output of the cell as the light was flashed on and off was noted. In a light adapted frog the output of a Class III cell is distinctive. The discharge pattern of the nerve pulses

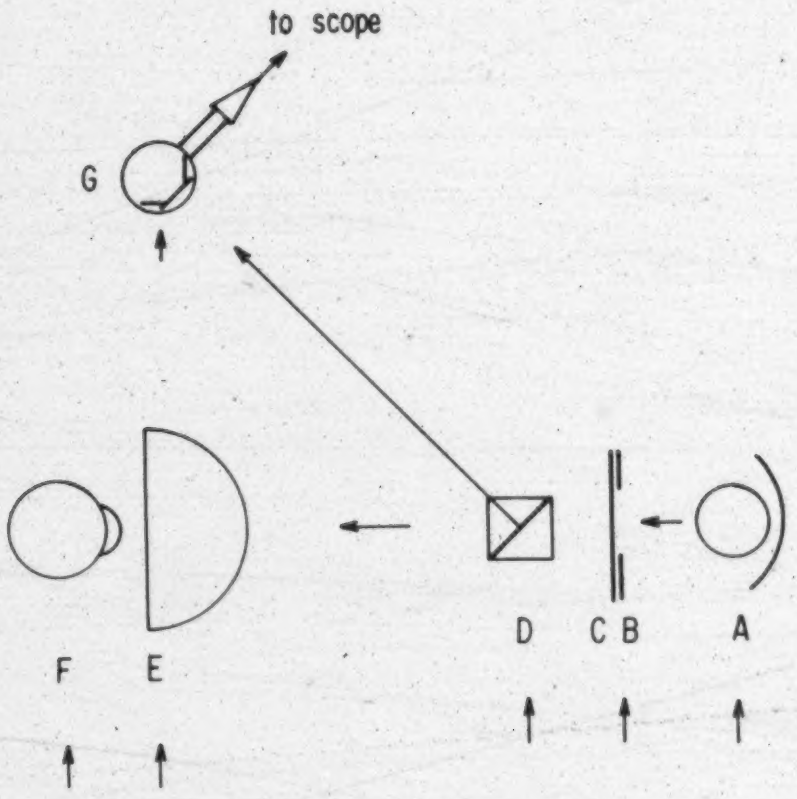


Figure 2. Schematic of light system for stimulus.

A - 12v. Automobile Lamp
B - Electronic Shutter
C - Neutral Density Filter
D - Beam Splitter
E - One-Half Ping Pong Ball
F - Frog's Eye
G - Phototransistor

consists of a few high frequency spikes at both the onset and again at the offset of the stimulus. While the latency of the On response is longer than the Off, the duration of the firing for both is approximately equal (Pickering and Varju, 1969). After a Class III cell had been found, the carbogen was turned on and the flow adjusted to 3 liters/min. The lights were extinguished and the frog remained in almost total darkness for 1 hour to dark adapt and completely recover from the anaesthetic. The only light in the room consisted of the pilot lamps on some of the equipment. These had been painted over to dim them. When recordings were made and recorded on a four-channel FM tape recorder, black paper was placed over the clear plastic shield over the reels. This eliminated light leaks. A 1.6 neutral density filter was placed over the counter to dim the light from this source. The small amount of light permitted through was used to read the stop watch used to time flashes longer than the 10 sec permitted by the shutter control box. It was also used to time the intervals between flashes. A shield was placed around the watch to reduce the light leaks. The oscilloscope screen, when in the store mode, produced a considerable amount of light. Two filters were mounted over the screen to reduce this source of light.

For those experiments in which recordings were made from the retina, a technique similar to that used in the tectal recordings was followed. The electrode was placed within the eye using the operation microscope and micromanipulator. The final placement was done by observing the electrode tip with an indirect ophthalmoscope and the use of the microdrive. Contact with the retina was observed

by an increase in the background noise. The same basic search pattern previously described was then followed. After the electrode was lowered to contact with the retina, flashes were given. The electrode was lowered with the microdrive. The flashes were repeated until a cell was located. If no cell was found, the electrode was raised and moved slightly. The procedure was repeated until a suitable cell was found. The frog was then allowed to dark adapt for 1 hour. One slight addition in the procedure occurred in the two series of experimental animals. The cryoprobe or heater wire was placed in position first and then the cell search began.

The cryoprobe was the standard Frigitronics Cryosurgery System with three modifications. The probe used nitrous oxide (N_2O) and was cooled by the Joule-Thompson effect. Since the instrument was designed to obtain temperatures of down to -89°C for cataract surgery and the experiments required temperatures in the range of -4 to +2°C, one adjustment was the reduction of the maximum pressure of the gas flowing to the probe. This was done at the factory by modifying the output of the regulator. The second modification was the placing of a small heating coil of 40-gauge teflon insulated constantan wire around the probe shaft and approximately 2 mm back from the tip. The coil used a No. 715, 7.5 v dry cell for a power supply. With the temperature regulation available from the control panel and the variable amount of heat which could be produced by the coil, via altering the resistance in the circuit, suitable temperatures were readily attained. The

probe also had a thermocouple mounted on it, at the factory, which gave accurate readings of the temperature of the probe tip. The probe shaft was insulated by tight-fitting plastic tubing. This prevented variations in the temperature of the probe caused by air currents or an occasional drop of water falling off the frog.

The heating coil consisted of a short piece of nichrome heater wire bent in the shape of a long U. A 12 v wet cell was used as the power source. A 2Ω, 50 watt rheostat was placed in the circuit to limit the current and prevent the melting of the wire.

Both the probe and heater wire were placed just in contact with the optic chiasma. Care was taken to avoid applying pressure on the optic nerve which might alter conduction through it.

After 1 hour had elapsed, the experiments were begun. The appropriate neutral density filter was placed in the holder and a test flash was given to determine if the cell was still present. Minor adjustments to the electrode position were made if required. In both the tectal and retinal controls, the filters were used in random order. The filter used had optical densities of 5.0, 4.3, 3.6, 3.2 and 2.6. According to the calibration of the light source and filters and the work of Fisher *et al.* (1970) the light stimuli covered the range from scotopic to the lower end of the photopic levels. The flash lengths used in the control series were randomly selected. Throughout all of the control experiments, a 1 min rest period was allowed between flashes. This enabled the frog to readapt. It was shown by Gordon and Graham (1973) that a 20 sec rest period was sufficient.

B. Flashes

The recording procedure during both series of control experiments was as follows. A flash was presented to the eye. The response was monitored on the oscilloscope. The response, phototransitor output, and a voice identification of the flash were simultaneously recorded on the FM tape recorder. The flash lengths used were 50 msec, 0.5 sec, 2 sec, 5 sec, 15 sec, 30 sec, 60 sec, 90 sec, and 120 sec. The sequence in which they were presented varied from day to day. The order the flashes were presented was as follows. The order either started with the shortest or longest flash and worked in order through the sequence. The next series of flashes was presented in the opposite order to the first series.

C. Block recordings

Before the reversible cold block experiments were performed, two preliminary experiments were done. The temperature for a reversible cold block had to be determined. Several workers (Boyd and Ets, 1934; Byck *et al.*, 1972; Douglas and Malcolm, 1955; Franz and Iggo, 1968; Gasser, 1931; Hodgkin, 1937, 1949; Paintal, 1965a, b) have studied reversible cold blocks on nerve of many species including the frog (Boyd and Ets, 1934; Gasser, 1931). There was some variability in the values reported; in fact, one worker (Boyd and Ets, 1934) noted that the most effective blocking temperature depended upon the temperature to which the frog had been acclimatized. Thus, a determination for the frogs used was performed. In the

first series of experiments, the sciatic nerve *in situ* was blocked with the cold probe. An electrical stimulus, just above threshold, was given above the level of the block and twitches in the gastrocnemius muscle were observed. The cold probe did block the nerve reversibly and the temperature range was similar to the findings of Boyd, i.e., -1° to +2°C. It was noted that the responses were better and could be repeated for a longer period of time if the nerve was allowed adequate time to recover from the cold. A second series of experiments was performed to provide an added check on the required temperature range. In this series the optic chiasma was reversibly cold blocked, as it would be in the later experimental series, but the recordings were made in the tectum. It was found that the nerve could be reversibly cold blocked in the temperature range of -4° to +2°C. This was the range of temperatures used in the cold block experiments. The variation was probably due to variability in the positioning of the probe relative to the chiasma.

The reversible cold block experiment procedure varied slightly from the controls. First, a few experiments were performed using the full range of filters and flash lengths. These were done for comparison with the controls. However, most of the experiments were performed at one intensity and duration of flash. These parameters had been determined during the control series. The actual recording procedure was as follows. A control flash of the fixed intensity and duration was presented and the response recorded. After 1 min the cold probe was activated. The cold probe was applied for 45 sec prior to the presentation of the stimulus and

remained on for the duration of the stimulus. The block was then released. The defrost cycle of the probe is 3 min. Two minutes in addition to the three for defrost were allowed for recovery. Another control flash was then obtained. One minute elapsed, and the same cycle of three recordings was repeated.

The procedure using the nichrome wire was similar. A series of a half dozen controls was recorded. The wire was heated. A recovery period of 10 min elapsed. Recordings were then made in the usual fashion.

At the end of all the experiments, controls, cold block, and nerve cutting, the frog was light adapted for a minimum of 15 min. A control flash of 2 sec duration was presented to the screen and the response recorded. This provided a check to ensure that a Class III cell had been used during the experiments. The cornea and retina were examined ophthalmically. Only data from frogs whose cornea was clear and had a brisk blood flow through the retina were used. Additional examinations were made on the cold blocked frogs and those whose chiasma had been severed. The optic chiasma was examined for the cold blocked frogs. Brisk blood flow through the small vessels around the chiasma was required if the data were to be used. In the other experimental series, the chiasma or optic nerve to the left eye had to be completely severed with no hemorrhage in order for the results to be accepted.

Another set of controls was performed to ensure that the results obtained were valid. First, it had to be determined that the cold probe was cooling the nerves and not either the blood flow to

the retina or the eye itself. It had been demonstrated (Branston and Fleming, 1968; Ecker, 1889) that in the frog no significant arterial blood supply is conveyed to the retina along or within the optic nerve. Thus, cooling of the blood was considered negligible and had no effect on the retina. While the cooling capacity of the probe is not great (Stumpf, 1972), a check was required to determine if the eye was being cooled. A thermocouple was placed under the skin as close to the exit point of the optic nerve from the eye as possible. Routine recordings were made. The thermocouple indicated no cooling of the eye. Temperatures of -20°C were also tried but no cooling effect on the eye was observed.

III. Data Analysis

After a reel of tape had been filled with data, it was replayed through a dual beam oscilloscope and photographed with an oscilloscope camera. The analysis of the film records consisted of the determination of the latency of the response to the onset and offset of the stimulus, the duration of each response, and the presence or absence of a rhythmic bursting firing pattern. The analysis also included the measuring of the burst durations and silent periods, in those records that displayed rhythmic activity. The interspike intervals were recorded from the films as well. The measurements were all performed by hand using a ruler. All intervals were measured to the nearest 0.5 mm. These values were then converted to seconds using the time marker on the film.

RESULTS

I. Tectal Recordings

The results of the first series of control experiments are based on 428 recordings from 18 frogs. The recordings were made from the terminal arbors of the optic axons in the third layer of the right statum album superficiale of the optic tectum (Maturana *et al.*, 1960).

The purpose of this series was to determine if changes occurred in the latency of the OFF response, duration of the response, or the firing pattern when the retina was subjected to stimuli of various intensities and durations. The results obtained followed the trend toward increasing latencies and longer durations of firing as observed by Pickering and Varju (1969). Figure 3 illustrates that the latency for short flashes is somewhat greater than for longer flashes. The figure also shows that there is little difference in the latency of the response for flashes of 5 sec and longer at the different stimulus intensities. Figure 4 indicates a similar trend for the durations of firing. Greater durations are seen for most of the short flashes. The durations then leveled off as the flash length was increased.

Another interesting observation was made. Within a narrow range of stimulus intensities and flash lengths when stimuli are presented to a previously dark adapted retina, a rhythmic bursting firing pattern appeared. The pattern was occasionally observed for the 30, 60 or 90 sec flashes using the 4.3 N.D. filter. The response

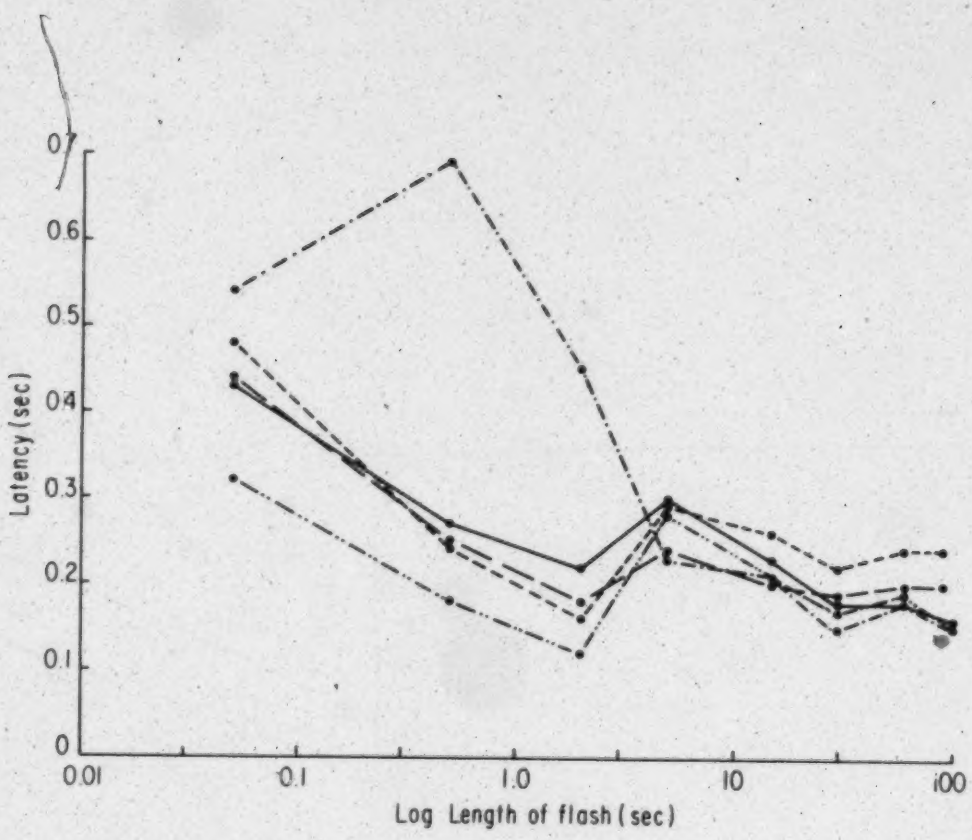


Figure 3. The latency of the Class III cell OFF response plotted against the log of the flash length. Recorded from the optic tectum. The standard errors of the means were deleted for clarity. The key is as follows: 5.0 N.D. filter -----, 4.3 N.D. filter — — —, 3.6 N.D. filter ————, 3.2 N.D. filter —'—'—'—', 2.6 N.D. filter —''—''

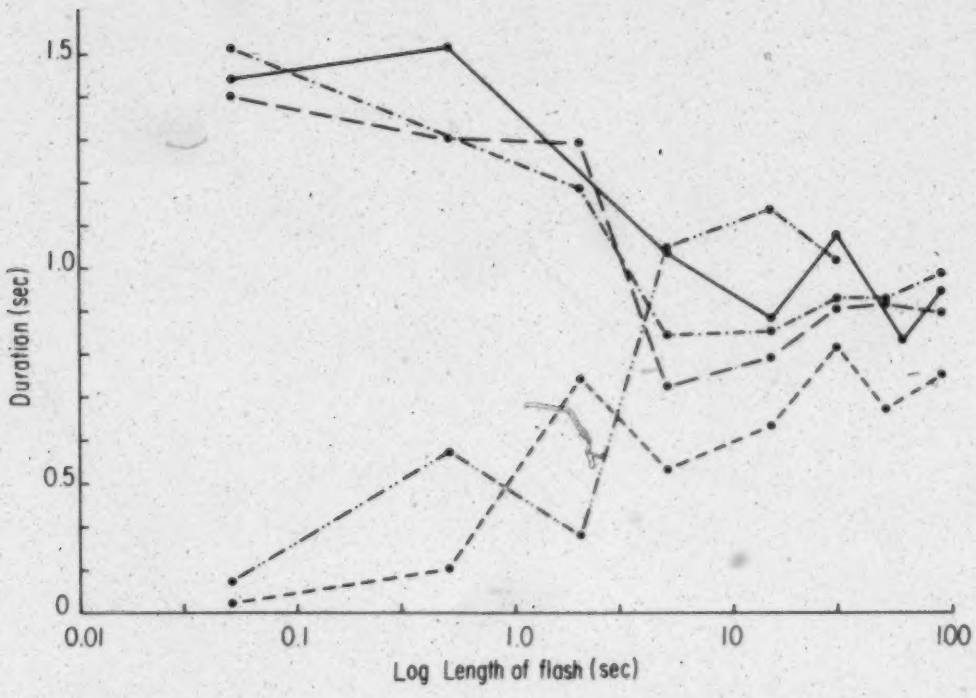


Figure 4. Duration of the OFF response plotted against the log length of flash. Recorded from the optic tectum. See Figure 3 for key.

was clearly seen in the 15, 30, 60 and 90 sec flashes using the 3.6 N.D. filter. It was occasionally seen for the longer flashes using the 3.2 N.D. filter. The pattern was rarely seen in the responses to the flashes using the 2.6 N.D. filter. The pattern was never observed for the very short flashes. In summary, this firing pattern was never observed for intensities above the 2.6 N.D. filter flashes nor below the 4.3 N.D. filter flashes no matter what flash length was used.

The calibration of the light source and filters indicated that the illumination presented to the eye when using the 3.6 N.D. filter was approximately 0.023 lux. This value falls into the mesopic range. Pickering and Varju (1969) found a change in the firing pattern which occurred at 0.07 lux. Birukow (1940) determined behaviourly that the mesopic level was 0.04 lux. These values are very close when compared to the total overall adaptation range of the retina. Thus, it seemed that a distinct firing pattern was generated by Class III cells when subjected to the offset of a background illumination that fell into the mesopic range.

II. Retinal Recordings

In order to confirm that the findings from the tectum were the true activity of the retinal ganglion cells and not solely a tectal phenomenon, extracellular recordings were made from Class III ganglion cells in the retina. (The work of Maturana *et al.* (1960) also demonstrated that the tectal activity was identical to activity in the ganglion cells.) The recordings were made extracellularly from

Class III ganglion cells in the retina. There are approximately 500,000 closely-packed ganglion cells in each frog retina (Maturana *et al.*, 1960; Maturana, 1958b). Thus, there is a large overlap of extracellular fields. This fact, in addition to the size of the electrode tips made it very difficult to completely isolate the response of one cell from those surrounding it.

The results of the retinal controls were based on 258 recordings from 11 frogs. The responses were similar to those obtained from the tectum. A Students t test was used to compare similar results from the tectum with those from the retina. In no case was a statistically significant difference noted. Thus, it was concluded that the responses recorded were the output of the Class III cells.

The same trend in the decrease in latencies for brighter flashes, seen in the tectum, was seen in the retina (Figure 5). The trend in the durations of the responses (Figure 6) was not as clear; however, as mentioned earlier, there was no statistically significant difference between similar durations of the response recorded from the tectum and the retina.

The data collected from the tectum and retina are summarized in Table 1. This is the first time that a study of the changes in the latency and duration of Class III cell responses has been made from scotopic to photopic levels. It can be seen that the Table shows little difference in either the latencies or durations of the responses. The rhythmic bursting pattern in the retina appeared at the same stimulus levels as in the tectal recordings. Since both

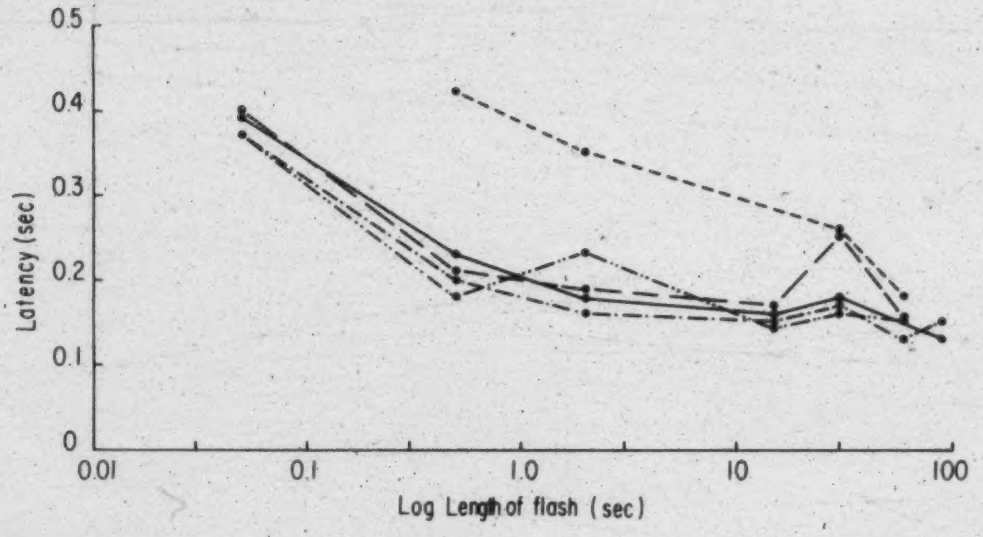


Figure 5. Latency of the OFF response elicited against the log length of flash. Recorded from the retina. Key: 5.0 N.D. filter -----, 4.3 N.D. filter — — —, 3.6 N.D. filter ————, 3.2 N.D. filter —·—·—·—·, 2.6 N.D. filter —··—··

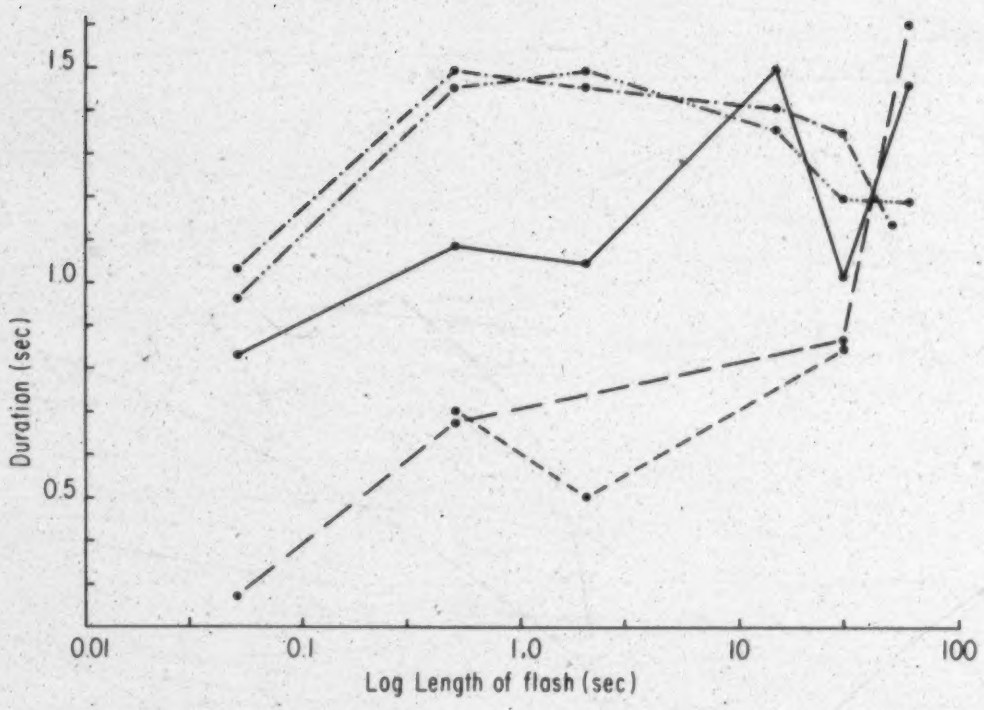


Figure 6. Duration of the OFF response plotted against the log length of flash. Recorded from the retina.

TABLE 1

Latencies and Durations of OFF Response

Controls ($\bar{x} \pm$ standard error)

Filter	Flash (sec)	Tect. Latency	Duration	Retina Lat.	Duration
5.3	5	.35	.58±.12		
	15	.35	.68		
	30	.34±.01	.53±.13		
	60	.37	.50		
	90	.33	.52		
5.0	.05	.48	.22		
	.50	.24±.01	.30±.03	.42	.70±.06
	2	.16±.03	.74±.16	.35±.04	.50±.15
	5	.29±.04	.53±.03		
	15	.26±.01	.63±.06		
	30	.22±.02	.81±.12	.26±.01	.84±.11
	60	.24±.02	.67±.05	.18	2.0
	90	.24±.02	.73±.05		
	120	.20	.65		
4.3	.05	.44±.01	1.4±.12	.40±.03	.27±.05
	.50	.25±.02	1.3±.09	.21±.02	.67±.08
	2	.18±.01	1.3±.09	.19±.01	.74±.04
	5	.24±.02	.72±.09		
	15	.20±.02	.79±.02	.17	
	30	.19±.02	.90±.09	.25±.02	.86±.10
	60	.20±.02	.91±.12	.15	1.59±.16
	90	.20±.06	.89±.47		
	120	.16	1.11		
3.6	.05	.43±.04	1.4±.09	.39±.01	.83±.04
	.50	.27±.01	1.5±.14	.23±.02	1.1±.12
	2	.22±.02	2.1±.13	.18±.01	1.0±.06
	5	.30±.03	1.0±.23		
	15	.17±.02	.88±.14	.16±.01	1.5±.01
	30	.18±.01	1.1±.11	.18±.01	1.0±.12
	60	.18±.01	.83±.12	.15	1.4±.07
	90	.16±.02	.94±.20	.13	1.6
	120	.13	.69±.20		

continued on next page

Table 1, continued

Filter	Flash (sec)	Tect. Latency	Duration	Retina Lat.	Duration
3.2	.05	.54±.02	1.5±.12	.37±.01	1.0±.05
	.50	.99±.13	2.7±.28	.20±.02	1.5±.06
	2	.45±.06	1.2±.04	.16±.01	1.4±.12
	5	.23±.05	.84±.14		
	15	.21±.02	.85±.21	.15	1.4
	30	.15±.01	.92±.12	.17±.01	1.3±.04
	60	.19±.02	.92±.11	.13	1.1
	90	.15±.02	.98±.10	.15	1.6
	120	.13	1.1±.02		
2.6	.05	.32	.27	.37±.01	.96±.05
	.50	.18±.10	.57±.13	.18±.01	1.4±.04
	2	.12±.01	.38±.03	.23±.05	1.5±.10
	5	.28±.06	1.0±.11		
	15	.21±.06	1.1±.24	.15	1.3
	30	.17±.01	1.0±.13	.16±.01	1.2±.05
	60	.19±.02	1.8±.30	.15	1.2
	90	.15±.01	1.8±.32		
	120	.11±.02	1.3±.60		

the retinal and tectal responses indicated that the mesoptic stimulus flash consistently caused the longest duration rhythmic response, it was decided to investigate this bursting phenomenon in more detail. This mesopic stimulus level was used in the experimental series investigating the possible efferent regulation of ganglion cell firing patterns. The other filter values were used in a restricted set of experiments in the scotopic and photopic regions for comparisons.

Figure 7 contains a sample of raw data recorded at three different stimulus intensities. All flashes used were 30 sec long. Trace A was recorded with a photopic flash. The next two traces, B and C, were recorded using a mesopic flash. Traces A, B and D were recorded from *R. pipiens* while trace C was recorded from *R. temporaria*. The rhythmic bursting firing pattern or periodic activity (Laufer and Verzeano, 1967) is readily observed. The last trace, D, was obtained using a scotopic flash. The difference in the firing patterns is readily seen.

III. Rhythmic Activity

Several properties of the periodic activity were examined. The analysis was divided into two parts: (1) single unit activity from one identifiable cell, (2) superimposed spike activity originating from groups of several cells.

A. Analysis of single units

Interval histograms of the interspike intervals from res-

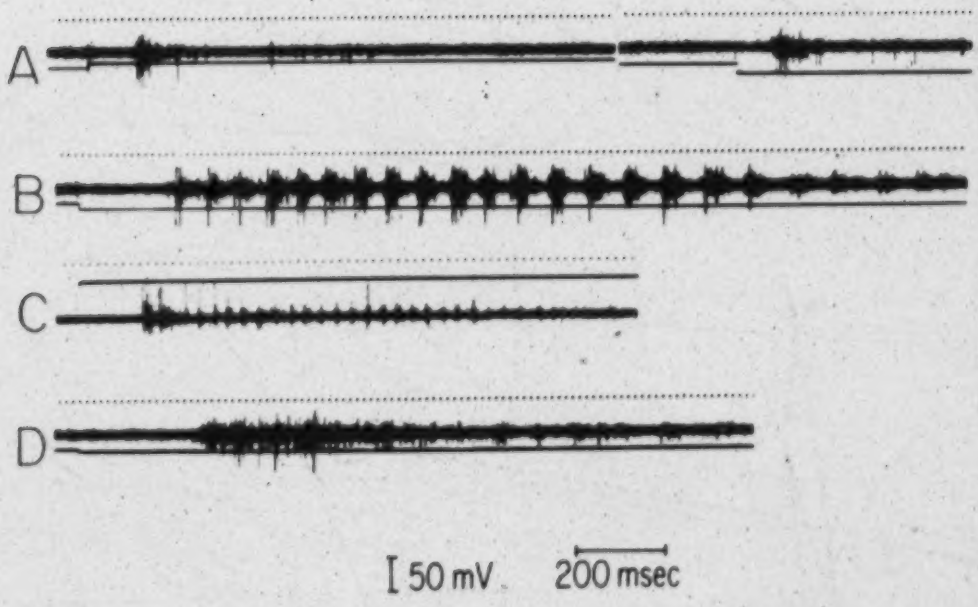


Figure 7. Sample recordings of the OFF response to three stimulus intensity levels. All flashes 30 sec long. Records A, B and D - *R. pipiens*; Record C - *R. temporaria*.

A. Flash from photopic level
B and C. Flash from mesoptic level
D. Flash from scotopic level

ponses recorded at three stimulus intensity levels are shown in Figure 8.These histograms display the interspike intervals observed at photopic, mesopic and scotopic stimulus intensity levels. It should be noted that the three histograms do not all represent the same number of recordings. The photopic histogram is based upon 25 recordings. The mesopic histogram is based upon 78 recordings, while the scotopic histogram is based upon 54 recordings. The figure shows that under photopic conditions, the response to the offset of the stimulus initiates spikes with short interspike intervals only. The mesopic stimulus also causes spikes with short interspike intervals; however, it also causes spike generation at markedly longer intervals. A close examination of the histogram indicates a clustering of the interspike intervals around certain values. The scotopic stimulus also causes spikes with short interspike intervals and to stimulate a few spikes with longer intervals; however, no clustering was observed.

Figure 9 illustrates this mesopic clustering phenomenon in more detail. For clarity, the interspike intervals of less than 0.02 sec are not plotted. The histogram indicates that some clustering of interspike intervals occurs around 0.048 sec, 0.096 sec, 0.144 sec and 0.192 sec. A regular pattern of firing seems to occur. The clustering around values which are multiples of the value 0.048 sec. It was observed, in the raw data, that cells sometimes fired in each and every burst of activity while sometimes they "skipped" or failed to fire in each one. Many records were observed where cells skipped one burst, thus, firing in alternate bursts. Others skipped two,

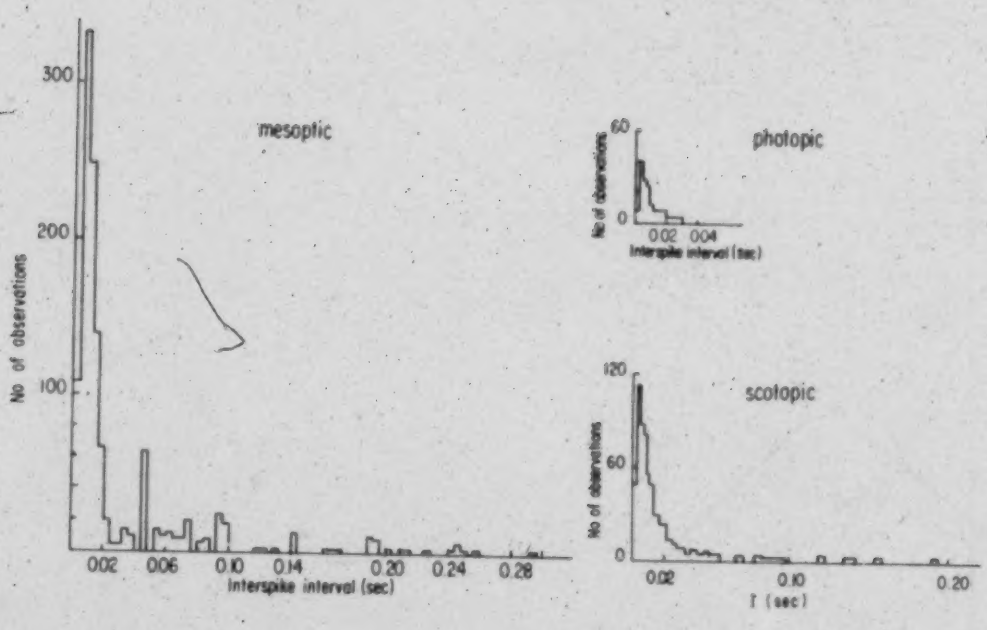


Figure 8. Interval histograms of interspike intervals at three different stimulus conditions.

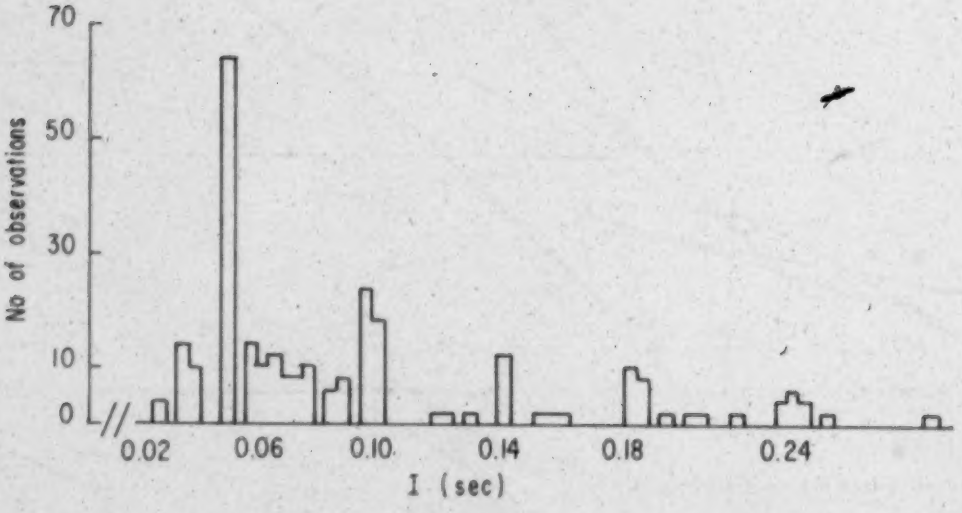


Figure 9. Interval histogram of interspike intervals excluding the very short values.

three or four bursts. This accounts for the clustering of interspike intervals around multiples of 0.048 sec interval.

It was desirable to determine if the tendency for a cell to skip was a function of the length of the response, i.e., was it more likely to skip at the beginning, middle, or end of the response. The peak around the 0.048 sec interval was called peak 1. The peak around the 0.096 sec interval was called peak 2, and so forth. The time of the occurrence of each observation in each peak was determined. The mean peak time was determined for each peak and plotted against the peak number. The result is Figure 10. This figure shows that the tendency for a cell to skip increased toward the end of the response.

Figure 11 examines in detail the interspike intervals of less than 0.02 sec. The time scale has been expanded in order to make the observing of the events easier. The histogram indicates that the cells are capable of firing with intervals as short as 0.002 sec. The most commonly observed intervals were those between 0.004 and 0.008 sec.

B. Analysis of multicellular spike activity

In many of the records, the electrode recorded activity from a number of cells in which the rhythmic activity then appeared as bursts of different amplitude spikes. The duration of each burst was examined. A burst was defined as the clustered responses of several cells (Laufer and Verzeano, 1967). In other words, the burst duration started with the first recognizable spike activity and

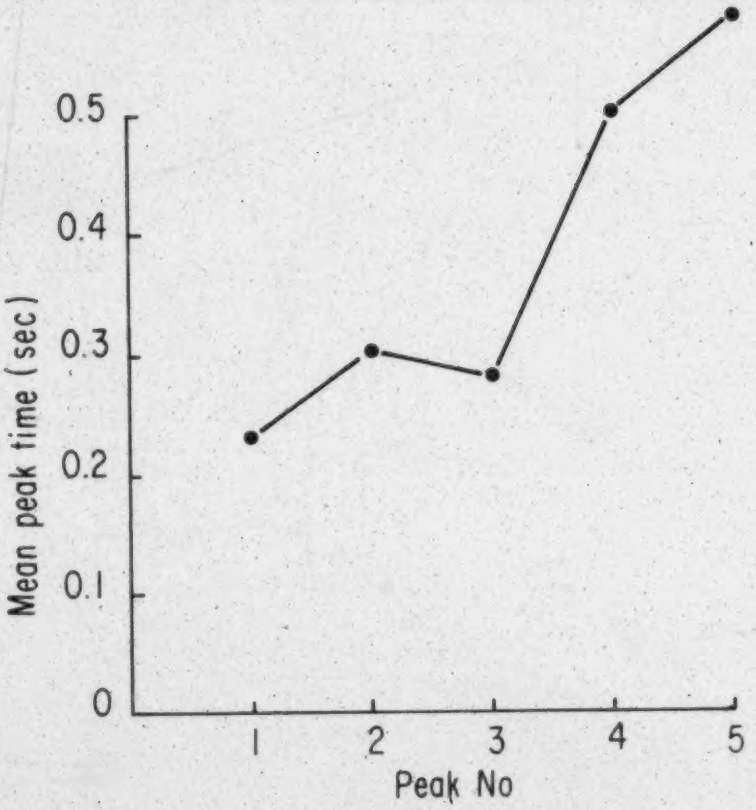


Figure 10. Plot of the mean time of "skipping" vs peak number.

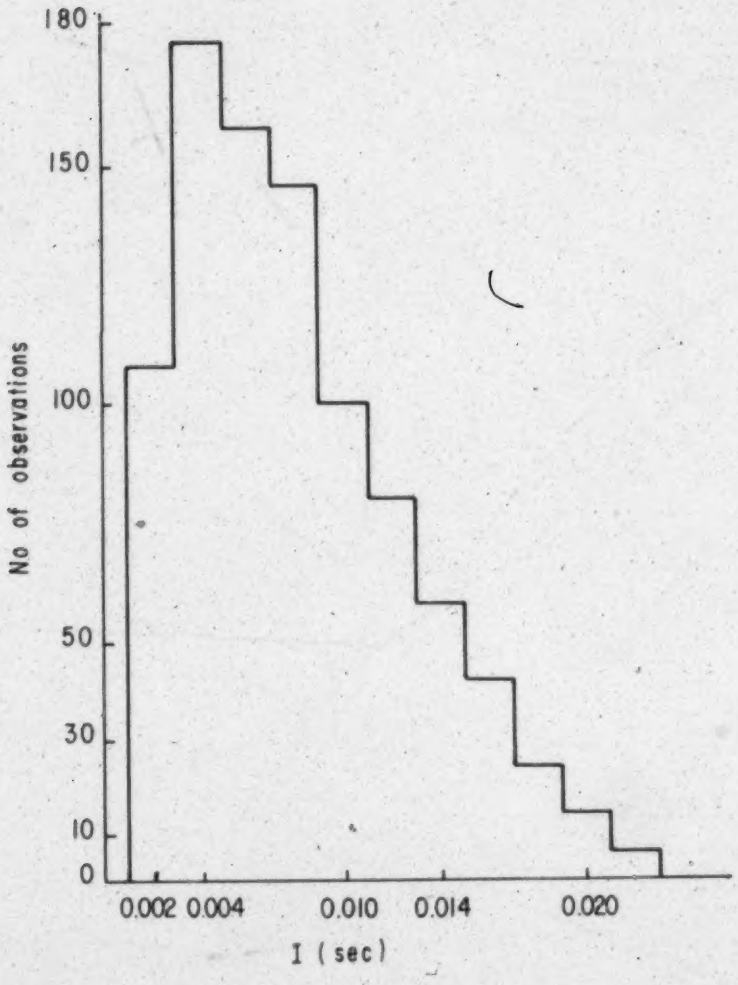


Figure 11. Interval histogram of the very short interspike intervals on an expanded time scale.

ended when no spike activity could be distinguished from the background noise.

Figure 12 is an interval histogram of burst durations. It can be seen that a burst duration of 0.048 sec was most commonly observed.

The silent periods were examined next. The silent period is defined as the period of inactivity between bursts of activity (Laufer and Verzeano, 1967). Figure 13 is an interval histogram of the silent periods. The mode was observed to be 0.028 sec.

Another piece of information was obtained from this data. It was found that an average burst frequency of 12.8 $\pm$ .3 bursts/sec occurred. This was determined by counting the number of bursts which occurred in 0.5 sec. The number was then doubled to give the number of bursts in 1 sec. The mean and standard error were then calculated. This average burst frequency cannot be calculated directly from Figure 9. Figure 9 does not consider the lengths of the silent periods. The time of occurrence within each burst that a particular cell fires is also not considered in Figure 9.

Some experiments were performed at stimuli slightly brighter than the stimulus used to obtain the above data. The mesopic stimulus was obtained using a 3.6 N.D. filter. The following data were obtained using a 3.2 N.D. filter. Some differences were noted. The most commonly observed burst duration was found to be 0.032 sec instead of 0.048 sec. The silent period mode was also found to be 0.032 sec as opposed to 0.028 sec for the above data. Figures 14 and 15 graphically illustrate these findings. An average

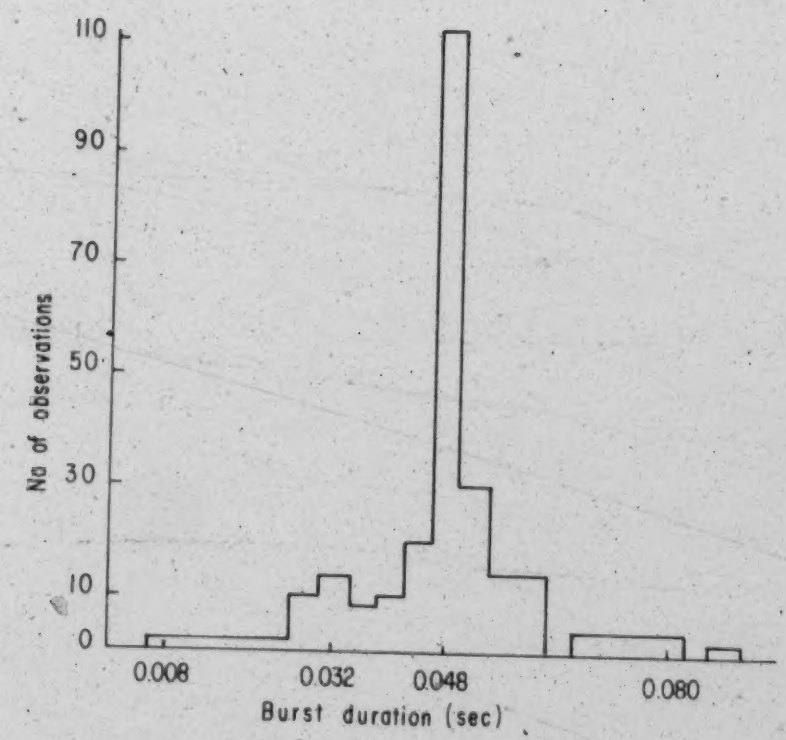


Figure 12. Interval histogram of burst durations. 3.6 N.D. filter, 30 sec flash.

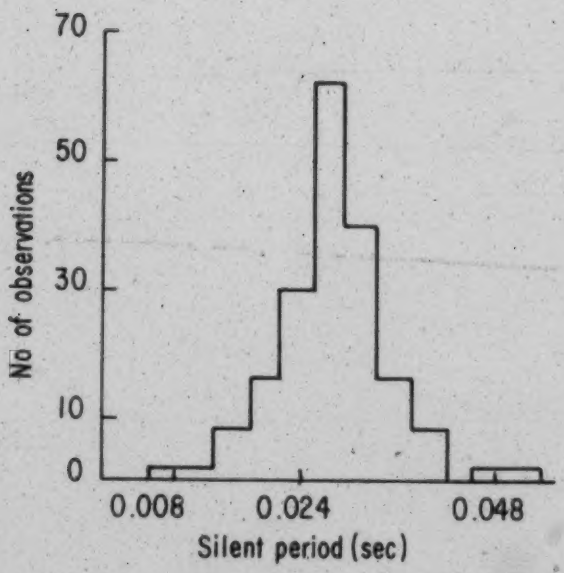


Figure 13. Interval histogram of length of silent periods. 3.6 N.D. filter, 30 sec flash.

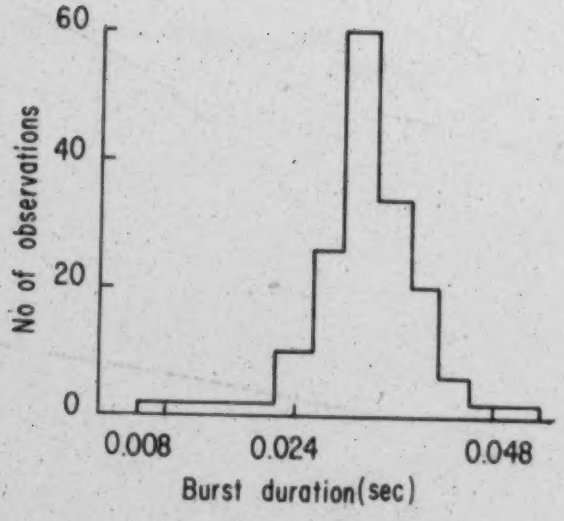


Figure 14. Interval histogram of burst durations. 3.2 N.D. filter, 30 sec flash.

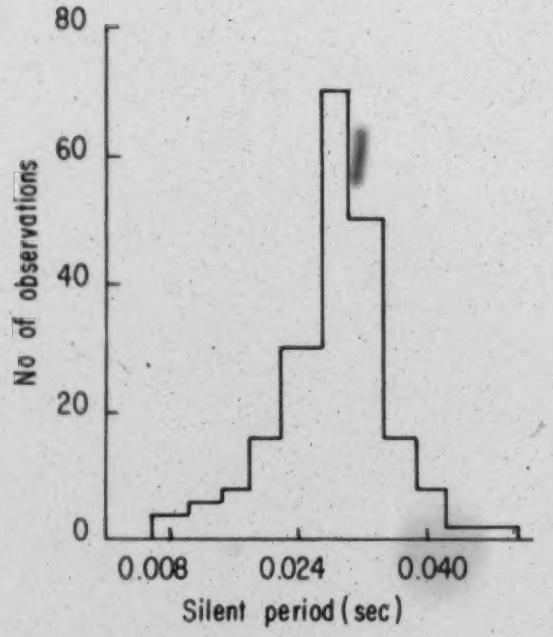


Figure 15. Interval histogram of lengths of silent periods. 3.2 N.D. filter, 30 sec flash.

bursting frequency was determined in the same manner as that for the above described results. The average bursting frequency was found to be a value of 15.6 $\pm$ .5 bursts/sec.

C. Comparison of data from different species

A few experiments had been performed using *Rana temporaria* instead of *Rana pipiens*. This data was analyzed in the same manner as that from *R. pipiens*. Figure 16 is an interval histogram of the interspike intervals. For comparison purposes, it was plotted on the same time scale as Figure 11. While Figure 16 is based on fewer records, the same trends are present as those in *R. pipiens*, i.e., most of the interspike intervals range between 0.004 and 0.008 sec.

Figure 17 corresponds to Figure 9. This figure illustrates, while excluding the data in Figure 16, that the interspike intervals cluster around the values of 0.032 sec, 0.064 sec, 0.096 sec, 0.128 sec and 0.16 sec, i.e., the events cluster around mutliples of 0.032 sec. This indicates periodic activity. As in *R. pipiens*, cells in *R. temporaria* also displayed some tendency to skip or not fire in each burst. The data were analyzed in the same manner as that used to prepare Figure 10. The data from *R. temporaria* produced Figure 18. The trend is the same in both species of frogs. The cells have an increasing tendency to skip in the later part of the response.

The burst durations and silent periods were also examined. Figure 19 shows that the mode of the burst durations is about 0.016 sec. Figure 20 shows that the mode, or most commonly observed, silent period is also 0.016 sec.

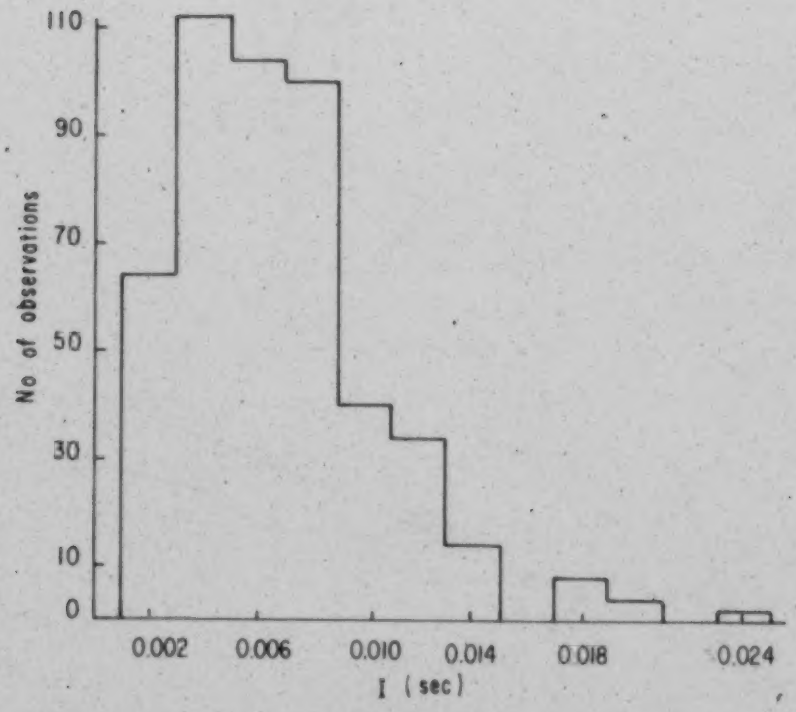


Figure 16. Interval histogram of the very short interspike intervals on an expanded time scale. *Rana temporaria*.

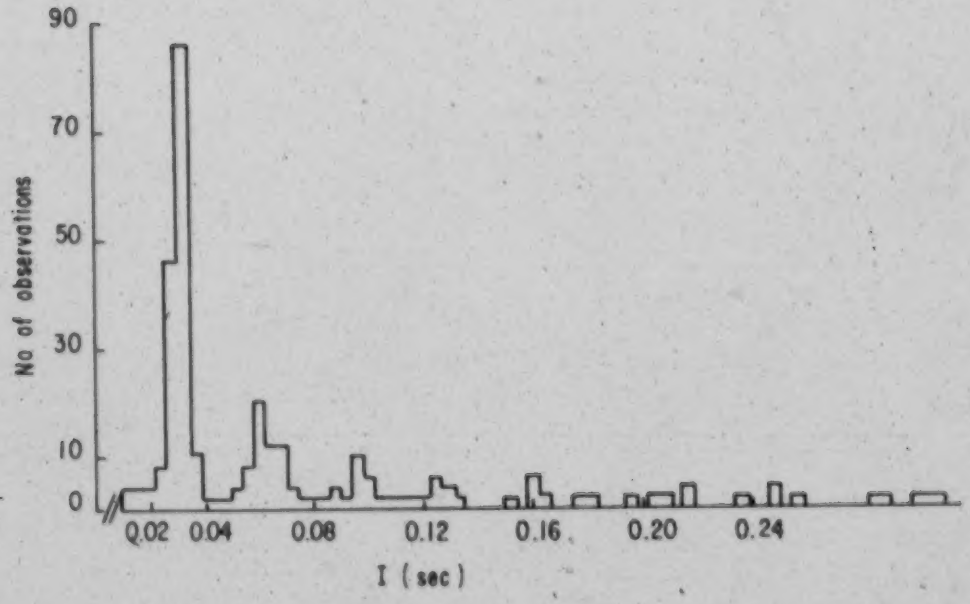


Figure 17. Interval histogram of interspike intervals. *Rana temporaria*.

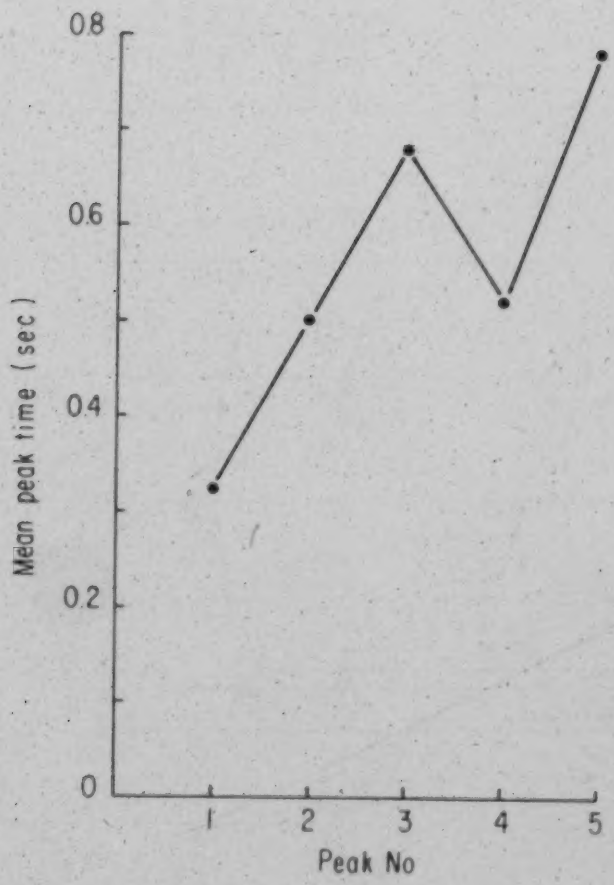


Figure 18. Mean peak time vs peak number. *R. temporaria*.

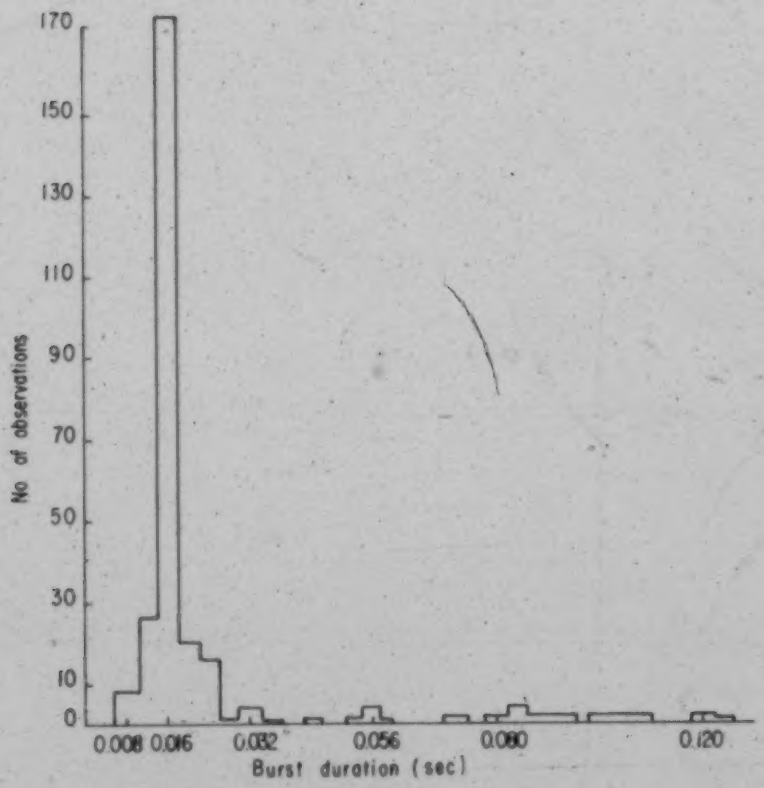


Figure 19. Interval histogram of burst duration. *R. temporaria*.

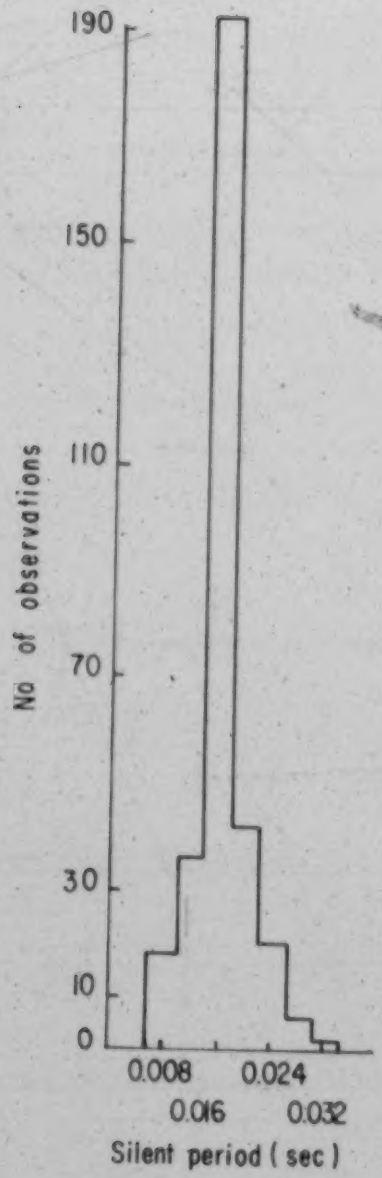


Figure 20. Interval histogram of silent periods. *R. temporaria*.

The average number of bursts/sec was found to be 31.2 $\pm$ .4. This is significantly larger than the 12.8 $\pm$ .3 bursts/sec observed in *R. pipiens*.

IV. Effects of Cooling the Optic Nerve

In order to test the hypothesis that efferent fibers to the retina might play a role in regulating ganglion cell activity, it was necessary to compare the output of the Class III cells with and without the efferent fibers. The results described above served as controls with an intact efferent system. They can be used for comparison with results from frogs which had the efferent system blocked. A cold block of the optic nerve at the chiasma could functionally isolate the retina from the brain. If efferent fibers had an effect on the output of the Class III cells, this may be altered. When the nerve was blocked, changes in the response would be noted. Before starting the cold block experiments, however, it was necessary to determine if a reversible cold block could in fact be obtained with the experimental procedure which was used. Figure 21 shows a recording made from the tectum with the cold probe in place at the optic chiasma. A 4.3 N.D. filter and 20 sec flash were used. The first response, C, was a control similar to those performed in the first series of experiments. Response B represents the response to an identical stimulus except that the cold probe was activated. The temperature of the probe tip was +2°C. The last response, C, is a normal control after the 5 min rest period. The test was performed 20 times on 2 frogs. Thus, a reversible cold block can be obtained

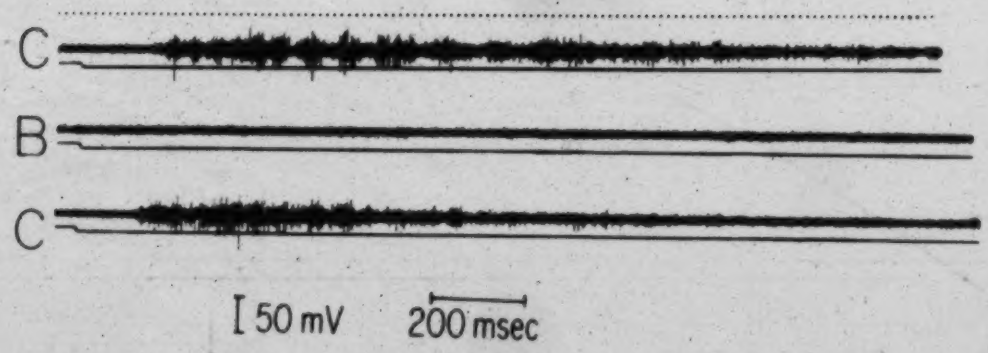


Figure 21. A reversible cold block of the optic nerve. Tectal recording. Temperature of tip +2°C. A 30 sec flash with the 4.3 N.D. filter. C. Control. B. Block.

at will.

When the experiments using the reversible cold block on Class III cells were performed, the following pattern was followed, i.e., one control run, one experimental run, and a repeated control run. The cold block results are based on 116 recordings from 11 frogs. At the same time, 179 responses without the cold block were obtained. Figure 22 illustrates the typical responses. The C responses are controls, identical to those done in the retina which have been previously described. The B responses are those to identical stimuli as used to obtain the C responses except that the cold probe was activated. Response A was a light adapted control performed to ensure that a Class III cell was used during the experiments. As can be seen from the figure, the rhythmic firing pattern disappeared and was replaced by a non-rhythmic mass of spikes similar to that observed for the scotopic flashes. This pattern was found consistently.

A comparison of the interspike intervals between the controls and the "cold blocked" animals is seen in Figure 23. The control graph is shown on top of the experimental one. However, the histograms contain an unequal number of recordings. More control data was available and was plotted. The ordinate scales have been made correspondingly different to better illustrate the data. The differences between the control and experimental responses are clearly seen as was shown in Figure 22. The histogram clearly illustrates the lack of bursting activity in the "cold blocked" animals. Most of the interspike intervals in the experimental

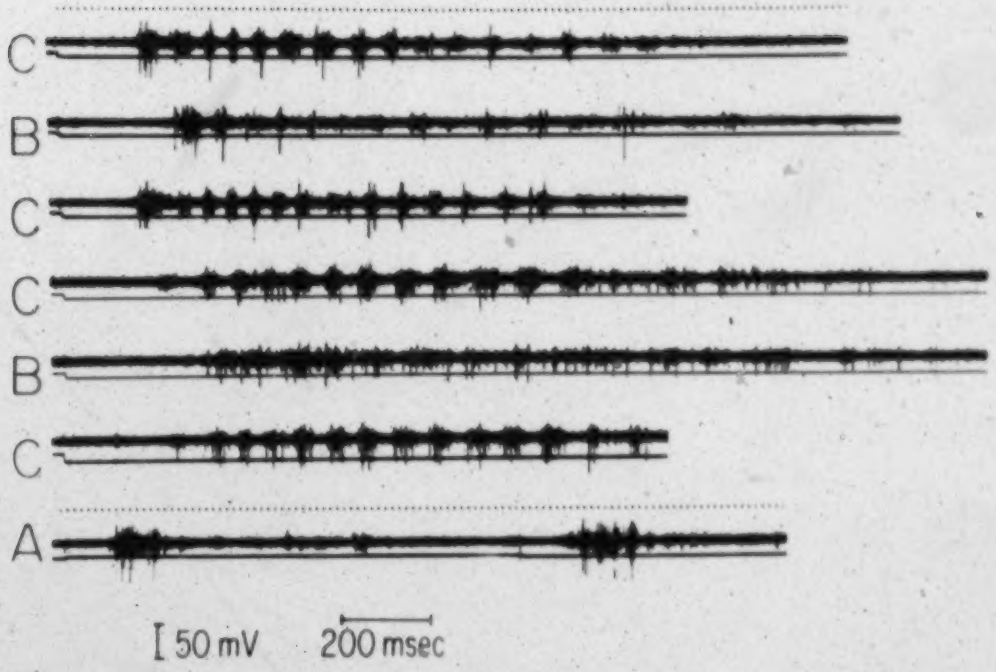


Figure 22. OFF response showing the effects of a reversible cold block applied to the optic nerve. All flashes were 30 sec long with 3.6 N.D. filter. Probe tip +2°C. C. Mesoptic Control. B. Cold Block (mesoptic). A. Light Adapted Control.

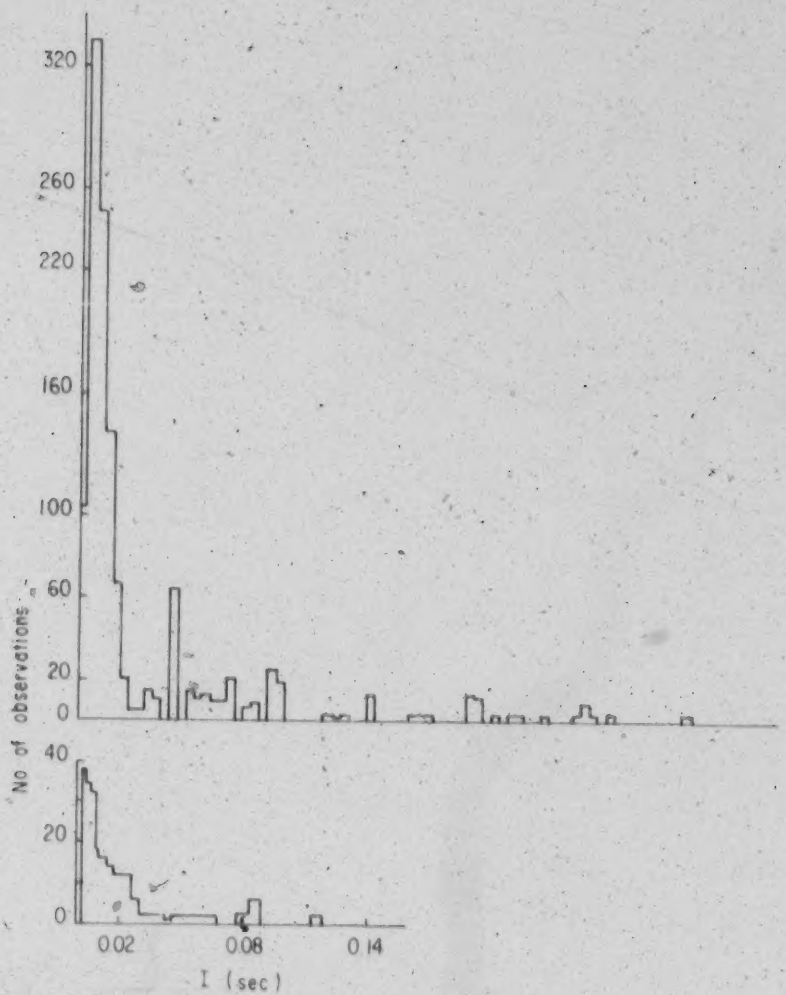


Figure 23. Interval histograms of interspike intervals at mesoptic level. Above: Control of normal intact preparation. Below: Cold block of the optic nerve.

recordings are less than 0.03 sec. Very few intervals are longer than this value. There is no clustering of the interspike intervals around a particular value or multiple of a particular value.

The controls recorded with the experimental studies were compared with the retinal controls. The Students t test indicated no statistically significant difference between the latencies or durations of similar flashes and filters.

Figures 24 and 25 graphically compare the latencies and durations of the responses observed with the cold block with the controls recorded at the same time. The Students t test indicated no statiatically significant difference between them. Thus, the only difference is the disappearance of the rhythmic bursting firing pattern.

In order to be certain that the nerve block was complete, a second series of experiments was performed. In this series a heater wire was placed just in contact with either the optic nerve or chiasma. A Class III cell was located and the frog dark adapted. After recording a few responses from the preparation, the wire was heated and severed the chiasma or nerve. After recovering from the severance, the same stimulus parameters were used. The results are based on 47 recordings from 4 frogs. Figure 26 illustrates the observed findings. Response A is a control. Responses B and C are typical responses from the frog after severance of the optic nerve. Response D is a light adapted control. This response served to confirm that a Class III cell had been used. It also showed that the frog would light adapt and appear to function normally after the

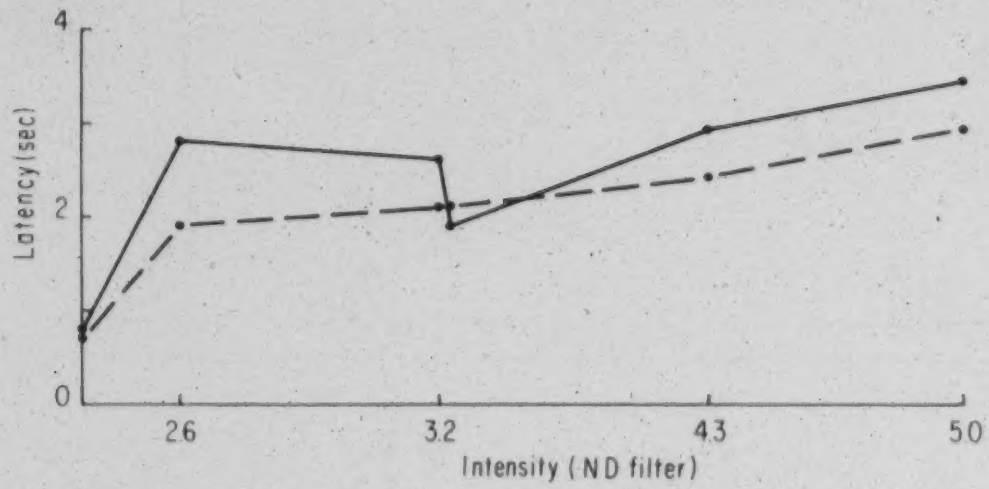


Figure 24. Latency of the OFF response plotted against the intensity of flash. Cold Block ———, Controls - - - - -.

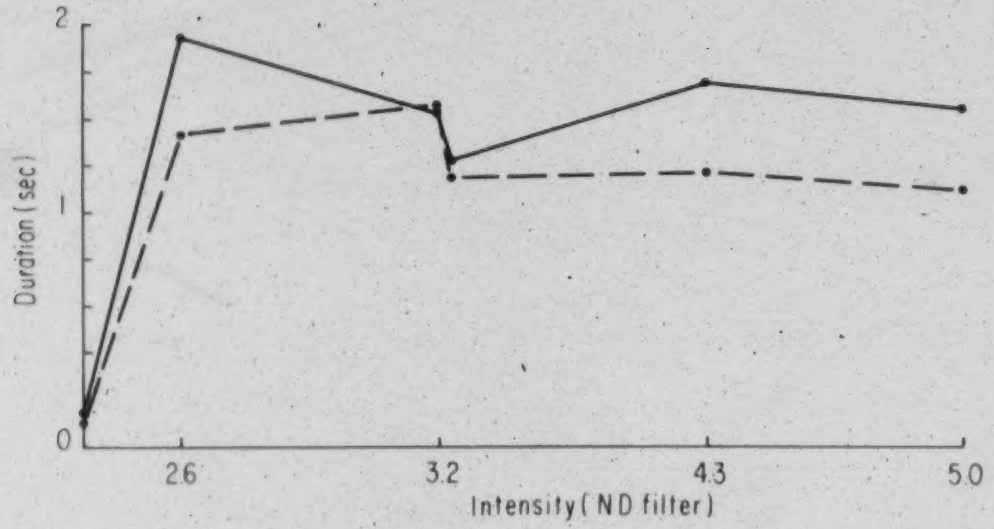


Figure 25. Durations of the OFF response plotted against the intensity of the flash. Cold Block ———, Controls -----.

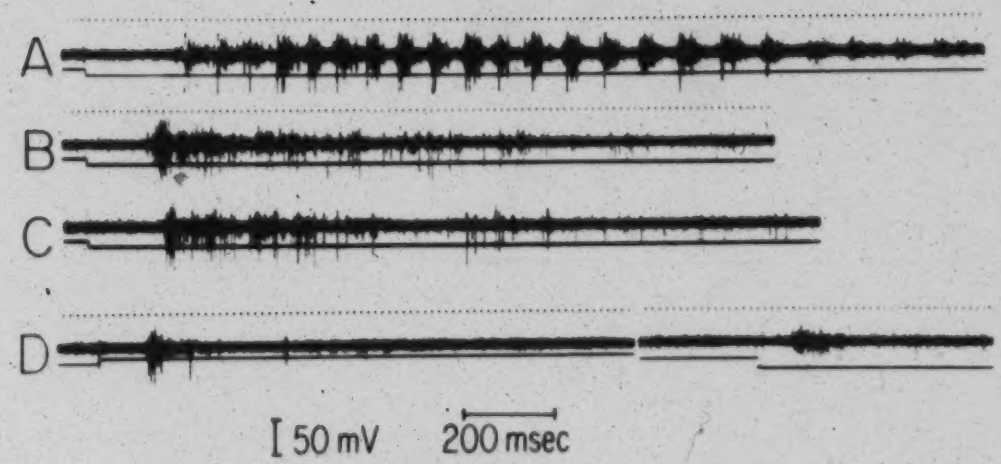


Figure 26. The OFF response from retina after severing the optic nerve. All flashes 30 sec long with 3.6 N.D. filter. A. Mesoptic control with nerve intact. B and C. Response after severing. D. Light Adapted.

retina had been isolated from efferent affects. As can be seen, the rhythmic pattern disappears upon severing the nerve; however, the Students t test indicated no difference with the latency or duration of the cold block results.

Table II summarizes the results obtained from the three series of control experiments as well as the cold block and cut nerve series. As mentioned earlier, no statistically significant difference was observed between the latencies and durations of responses from similar flash lengths and intensities. The only difference was the disappearance of the rhythmic activity in the blocked and severed nerves.

Table III summarizes the data collected on the ON response in the mesoptic range. No significant change was noted in the ON response. Table IV indicates that no large change occurred in the light adapted responses either. It was noted, however, that the latency of the light adapted ON response was slightly larger in the "severed" preparation than in the control preparations. This was the only observed difference in the data listed in the Tables.

V. Summarizing the Results

It has been shown that under certain conditions, a rhythmic firing pattern appears in response to a certain restricted range of stimuli. This stimulus is the offset of illumination in the mesoptic range. This particular firing pattern has not been described previously in the frog. Further, it has been shown that efferent fibers play a role in the rhythmic activity. The removal of the efferent influence,

TABLE II

Latencies and Durations of OFF Response

Comparison of all Controls with Experimental ($\bar{x} \pm s_{\bar{x}}$)

30 sec flashes

Filter	Exp. Lat.	Exp. Dur.	Controls done with Exp. Lat.	Controls done with Exp. Dur.	Retina Cntl. Lat.	Retina Cntl. Dur.	Tectum Cntl. Lat.	Tectum Cntl. Dur.
5.0	.34±.05	1.4±.26	.29±.03	1.1±.21	.26±.01	.84±.11	.22±.02	.81±.12
4.3	.29±.02	1.6±.15	.24±.01	1.2±.14	.25±.02	.86±.11	.19±.02	.90±.09
2.6	.19±.01	1.2±.04	.21±.01	1.1±.03	.19±.01	1.0±.13	.18±.01	1.1±.11
3.2	.20±.05	1.4±.05	.21±.02	1.5±.13	.17±.01	1.3±.04	.15±.01	.92±.12
2.6	.28±.06	1.7±.29	.19±.03	1.3±.10	.16±.01	1.2±.05	.17±.01	1.0±.13
L.A.	.80±.006	.14±.02	.07±.004	.09±.01	.07±.001	.10±.03	.08±.009	.12±.07

TABLE III

Latency and Duration of ON Response

Retina Controls vs Block

3.6 N.D. Filter

$\bar{x} \pm s_{\bar{x}}$

Controls		Block	
Latency	Durations	Latency	Duration
.24±.01	.26±.02	.26±.01	.29±.03

TABLE IV

Latency and Duration of Light Adapted ON and OFF Responses

Retinal Controls vs Cut Nerve

	ON		OFF	
	Latency	Duration	Latency	Duration
Control	.07±.005	.09±.01	.07±.007	.11±.13
Cut	.10±.002	.09±.01	.08±.006	.14±.02

via the cold block or nerve severance, eliminated the rhythmic activity. In the case of the cold blocked experiments, the rhythmic activity returned after the nerve had returned to room temperature.

DISCUSSION

The results of the above described experiments can now be compared with earlier work described in the literature. It can also be discussed in regard to the possible significance of the rhythmic activity and the function of the efferent fibers. One possible model, describing how the rhythmic responses may be generated, is also developed.

Laufer and Verzeano (1967) described rhythmic activity in the visual system of the cat. They found that the ganglion cells fire in bursts separated by silent periods of inactivity. They further found that there was little variation in the size of this silent period, "interburst interval". The occurrence of the bursts of multineuronal spikes were found to be highly rhythmic. In the experiments studying the occurrence of the rhythmic activity when the stimulus was extinguished, the bursts were found to occur at frequencies of from 30-40/sec. In the present experiments on *R. pipiens* and *R. temporaria*, similar rhythmic activity was observed. The ganglion cells were found to fire in bursts which were separated by silent periods. It was also observed that there was little variation in these interburst intervals.

Laufer and Verzeano (1967) suggested that the centrifugal fibers to the retina might influence the rhythmic activity, but were not sure if they caused it. However, they did speculate that the rhythmic activity was implicated in "the mechanisms which regulate the level of responsiveness of the retina in relation to the conditions

of light and darkness in the enviromment". This conclusion supported Granit (1941), who recorded discharges consisting of groups of spikes in the frog retina in response to the onset and cessation of light. Granit also felt that this activity regulated retinal sensitivity.

Tomita and Fumaishi (1952) and Brindley (1956) studied the oscillatory potentials in the bullfrog and frog retina respectively. These workers found that the amplitude of the oscillations was largest in the area of the bipolar and amacrine cells. These workers did not speculate whether these cells themselves initiated the rhythm or if an input to them could have caused the oscillations.

Doty and Kimura (1963) studied the oscillatory potentials in the optic nerve of the cat and monkey. These workers used both gross and microelectrodes in their work. These workers speculated that, on the basis of their results, efferent fibers, ending around the amacrine cells, were probably the source of the rhythmic potentials.

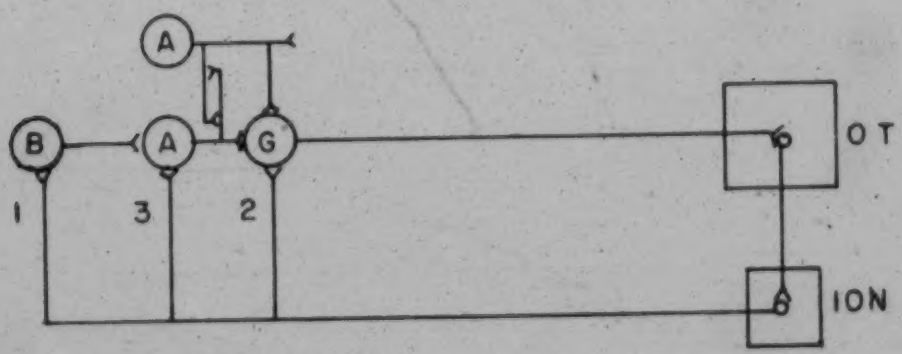
Several workers (Volkmer, 1956/57; Granit, 1933; Yonemura *et al.*, 1963; Yonemura and Hatto, 1966) studied the effect of different light intensities on the frequency of the rhythmic response. It was found that the light intensity did not affect the frequency of the oscillations. In the present work, the change from a 3.6 N.D. filter to a 3.2 N.D. filter caused a shortening of the burst duration and a lengthening of the silent period. A slightly longer frequency was also observed. This indicates that the rhythmic activity is, in fact, affected by the light level. A slight elevation in the stimulus intensity increased the frequency to a new value. This is in contrast to the situation observed by others.

Efferent fibers have been reported in the frog (Branston and Fleming, 1968; Cajal, 1952; Maturana, 1958b) and pigeon (Cowan and Powell, 1963). Miles (1970, 1972a, b, c, d) and Rogers and Miles (1972) studied the efferent fibers to the chick in detail. Miles found that the efferent fibers seemed to have a disinhibitory function to the surrounds of the RFs. Rogers and Miles (1972) also found that the efferent fibers also seemed to play a role in the adapting of the retina to different background illumination levels.

Doty and Kimura (1963) and Laufer and Verzeano (1967) have suggested that rhythmic or periodic activity is due to some inhibitory input to the ganglion cell. Algvere and Wachtmeiser (1972) also attributed the rhythmic response to be the result of inhibitory modulation of a cell's response. The ganglion cells have been shown, in the present work, to have been able to fire spikes with inter-spike intervals as short as 0.002 sec. The most commonly observed intervals were found to be between 0.004 sec and 0.008 sec. Yet, intervals as long as 0.048 sec and multiples of this value were observed in the rhythmic response. The largest multiple of 0.048 sec observed was five times this value. Thus, it appears that since the cells are capable of firing at a much higher frequency, there must be an inhibitory input which modulates the output of the ganglion cells. In the experiments in which the retina was functionally isolated from the brain, the rhythmic response disappeared. Thus, if Doty and Kimura (1963), Laufer and Verzeano (1967) and Algvere and Wachtmeiser (1972) are correct, then these results indicate that a source of inhibition has been removed.

Thus, it can be seen that the observed rhythmic activity may be due to the modulation of the ganglion cell output by efferent fibers. It has also been suggested by the previously cited workers that this activity could be related to the regulation of retinal sensitivity.

It can be seen that the efferent fibers and/or amacrine cells have been implicated in initiating rhythmic activity. Several models as to the location of the source of the rhythmic activity are possible. The following schematic diagram illustrates the currently best known anatomical pathway.



B. Bipolar cell

A. Amacrine cell

G. Ganglion cell

OT. Optic tectum

ION. Isthmo-Optic Nucleus

As cited previously, Lazar (1969) has shown the existence of the tecto-isthmo-optic pathway in the frog. Larsell (1924) has demon-

strated, in the frog, that the efferent fibers from the ION join the optic nerve at the chiasma and travel to the retina. Rubinson (1971) confirmed the existence of the tecto-isthmo-optic pathway. The efferent fibers synapse with the amacrine cells (Cajal, 1952). Dowling (1968) found no evidence to support the paths labeled 1 or 2. No synapses between bipolar cells and efferent fibers, nor between ganglion cells and efferent fibers were observed. However, extensive synapses were associated with amacrine cells. Serial synapses between 2 and 5 amacrine processes were commonly observed. Some reciprocal synapses were found between two amacrine cell processes. It is possible that the efferent fibers could be one of the processes in the series.

The source of the rhythmic activity could be in either one of two places. The first could be the retina. Some evidence exists to support this view. Barlow (1953) observed rhythmic potentials, in the isolated eyecup preparation of *R. temporaria*, as the retina dark adapted. The ganglion cells were observed to fire in groups of impulses with a "speed" of 90/min. He also observed that if several cells were recorded from, the units "joined" and "left the rhythmic discharge". Pickering (1973) also observed some rhythmic potentials in an isolated retina, but infrequently. Thus, one possible source of the rhythmic responses could be within the interconnections of the amacrine cells.

However, several problems with the data exist in Barlow's work. No successful attempt was made to keep the preparation moist or supplied with oxygen. He observed that the rhythm appeared in

damaged preparations or ones which were drying out. He also observed that his preparations would become unresponsive after varying lengths of time. Thus, the condition of his preparations is questionable. Also, the rhythm Barlow observed had a frequency of 90/min. The rhythmic activities also recorded in the present work had much higher frequencies. Further, it was observed in the present work that all the cells recorded from at one time were firing synchronously; Barlow indicates that the ones he recorded from may not have been.

The results obtained by Pickering were found in only a few cases. Light adapted controls were performed; however, the responses were not similar to normal light adapted responses. Also, a response was observed only for stimuli with durations greater then 15 msec.

The experimental results, described in the previous chapter, used both an optic nerve cold block and optic nerve severance. The severance was performed on a dark adapted frog. The heating of the loop of heater wire to cut the optic nerve did not affect the electrode placement. The preparations were supplied with an enriched oxygen atmosphere and kept moist. A total of 163 recordings were made. In no case was a rhythmic response observed after isolation of the retina. A response was obtained from stimuli of 0.5 sec. Upon light adaptation, a normal response was obtained from the preparations whose optic nerve had been completely severed. Thus, a serious doubt exists whether or not the ganglion cells in the isolated retina are capable of firing with rhythmic activity.

A second possible location for the source of this rhythmic

activity could be either in the ION or some other region in the central area. This suggests that the efferent fibers regulate the amacrine cell responses and effect the rhythm. The efferent fibers must either be excitatory or inhibitory to the amacrine cells, depending upon whether or not the amacrine cells are usually excitatory or inhibitory to the ganglion cells. It is possible that the amacrine cells are only inhibitory; however, it is not likely that a continuously inhibitory cell exists as the sole input to the ganglion cells. The amacrine cells could be excitatory. They could also be a combination of both. The response of one particular amacrine cell would probably be determined by the sum of the inputs to it from bipolar cells and other amacrine cells. This makes the determining of the nature of the efferents difficult from the extracellular recording techniques which have been used. If the amacrine cells are usually excitatory, the efferents could provide a rhythmic inhibitory input to the amacrine cells and effect the rhythm. Severance of the optic nerve would then release the amacrine cells from an inhibitory input and the non-rhythmic response could occur.

The time required for a response initiated by a ganglion cell to go around the anatomical pathway cannot be calculated precisely. The conduction velocity of the Class III cells of *R. pipiens* has been determined by Maturana *et al.* (1960). The time for the response to appear in the ION and the conduction velocity of the efferent fibers has been determined in the pigeon (Holden, 1968b; Galifretet *et al.*, 1971). The response time of the amacrine cells has been observed in the isolated eyecup of *Necturus* (Werblin and

and Dowling, 1969). Circuit times of from 0.02 sec to 0.07 sec are possible. Thus, information about the anatomical circuit time constants does not provide much useful information for pinpointing the source of the rhythmic activity.

The cold block and nerve severance experiments demonstrate that the rhythmic response disappears when the retina is functionally isolated from the brain. However, the rhythmic response returned after the nerve warmed up to room temperature. This could mean that the source of the rhythmic activity is in the brain and rhythmically inhibits the amacrine cells.

However, the data of Barlow (1953) and Pickering (1973) should not be totally disregarded. Thus, on the basis of the present experiments, it cannot be stated conclusively where the source of the rhythmic activity is located. The present data appear to suggest a central origin. All that can be concluded is that the efferent fibers do affect the firing pattern of Class III ganglion cells.

Another observation made was that of the cell's "skipping" a given burst. This could be due to the failure of a particular amacrine cell or a group of amacrine cells to reach the level of depolarization required to stimulate the ganglion cell to fire. Often, however, the adjacent ganglion cells (in the background) continued to fire. It was observed that the other ganglion cells, recorded in the background by the extracellular electrode, were synchronized and fired within the same bursts. This synchronized firing within each burst could be due to rhythmic inhibition of many of the amacrine cells by the efferent fibers.

The failure of one cell to fire in a burst does not interrupt the chain of input to the optic tectum and feedback to the retina from the ION. Other cells continue to fire. In no case was a completely skipped burst observed, i.e., no activity at all when a burst was expected. However, even if this had been observed, it does not necessarily indicate a breakage of the loop. Other ganglion cells in areas beyond the pickup range of the electrode could be continuing to fire, thus maintaining the input to the optic tectum and the feedback to the retina. No physiological evidence has been obtained which clearly demonstrates the nature of the interactions between the bipolar and amacrine cells nor among the amacrine cells. It is speculated that the skipping could be due to a retinal process. This could be obtained through the serial synapses among the several amacrine cells. The data obtained from the functionally isolated retina does not provide conclusive evidence for either view. The very short interspike intervals observed in the functionally isolated retina were similar to those in the control experiments with only a slight tendency to longer values. However, the responses were not rhythmic, but appeared random, that is the cells did not fire at regular interspike intervals. This might suggest a retinal process for controlling a ganglion cell's "skipping" response, but is not conclusive proof.

In summary, it had been demonstrated that the OFF response within a small restricted range of stimulus intensities initiated a rhythmic response. This occurred primarily at the offset of a mesopic stimulus. When the efferent fibers, within the optic

nerve, were blocked, this rhythmic activity was transformed into a non-rhythmic random pattern. It was concluded that the efferent fibers in the optic nerve play a role in the regulation of the retinal ganglion cell output.

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